# Freshwater Mussel Assemblages of the Wolastoq | Saint John River, New Brunswick: Establishing Baseline Population Metrics and Habitat Associations

by

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## ABSTRACT

Freshwater mussels are among the most imperiled animals in the world and continue to experience reductions in distributions and range, and the loss of species from communities. As keystone species and ecosystem engineers, they provide ecosystem services that benefit both the surrounding freshwater environment, e.g., protecting, and sustaining ecosystem functions and water quality. Surveys for freshwater mussels are used to learn about the status of mussel populations and the health of aquatic ecosystems, yet there is a lack of current and historical data for freshwater mussel populations in most of Atlantic Canada. Baseline population data is becoming increasingly important amid threats such as continued anthropogenic pressures, invasive species, and climate change. I present the results of extensive freshwater mussel surveys throughout the Wolastoq | Saint John River, New Brunswick, the river with the greatest freshwater mussel diversity in the Maritimes. These surveys help to establish baseline population metrics for the Saint John River and allowed me to determine associations between freshwater mussels and their physical habitat. This baseline information can be used to help better understand the basic ecology of the freshwater mussel community as well as guide freshwater mussel management, conservation, and future freshwater mussel research efforts in the Wolastoq | Saint John River, New Brunswick, and Atlantic Canada.

## DEDICATION

This thesis is dedicated to everyone who has supported my academic endeavors. I will be forever thankful for the endless support, patience, and wisdom you lent over the course of my studies. "Nothing great was ever accomplished without making sacrifices"

- Anonymous.

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# Chapter 1 - General Introduction

Freshwater mussels (Bivalvia; family Unionidae) are a group of aquatic mollusc inhabiting freshwater systems and are widely considered keystone species and ecosystem engineers as they modify, create, and maintain the health and stability of habitats to the benefit of themselves and other organisms (Gutiérrez et al., 2003; Spooner & Vaughn, 2006; Vaughn, 2010; Spooner & Vaughn, 2012). They often dominate the benthic biomass in lakes, rivers, and streams where they play fundamental roles in their ecosystems as filter feeders that store phosphorus and nitrogen, oxygenate sediments, and connect pelagic and benthic systems (Parmalee & Bogan, 1998; Nedeau et al., 2000; Martel et al., 2010). Freshwater mussels have many characteristics and life history traits that make them ideal bioindicators of aquatic health which include a long lifespan, a sedentary filter-feeding lifestyle, a sensitive larval stage, and their use of a vertebrate host (typically fish; Naimo, 1995; Angelo et al., 2009). Despite the important services that freshwater mussels provide to ecosystems, they are often overlooked when studying freshwater organisms and environments.

Unfortunately, this ecologically important group is considered one of the most imperiled groups of organisms on earth (Haag & Williams, 2014; Collier et al., 2016). In North America, roughly 60 taxa have been lost in the past century, and 65% of the species remaining are considered endangered or at risk (Haag & Williams, 2014). Freshwater mussels continue to experience loss of diversity, decreases in abundance, and reductions in distribution and range. The decline, extirpation, or extinction of freshwater mussel populations will impair ecosystem function and negatively affect water quality as well as other aquatic organisms that benefit from their services (Vaughn et al., 2004; Collier et al., 2016).

The catastrophic decline of freshwater mussel populations is likely due to their sensitivity to habitat alteration (Haag, 2012; Haag & Williams, 2014). The mass construction of impoundments and the channelization of river systems during the 1920s to 1980s is believed to have had the greatest negative impact on freshwater mussel populations to date (Bogan, 1993; Haag & Williams, 2014). The dams constructed during this period are considered responsible for many known freshwater mussel extinctions (Haag, 2012; Haag & Williams, 2014). The construction of a dam creates an upstream reservoir with lake-like conditions and a highly variable downstream reach that can be more characteristic of headwater-like conditions in some cases (Vaughn & Taylor, 1999; Gangloff et al., 2011). Freshwater mussel populations are flooded in reservoirs and washed away or buried in tailwaters. Dams and impoundments can cause seasonally depressed water temperatures, frequent and irregular fluctuations in flow and water levels, as well as changes in sediment and nutrient transport, and substantial channel scour (Baxter, 1977; Poff et al., 1997). The dam itself is a barrier to the upstream movement and migration of fish hosts, which freshwater mussels require for successful reproduction and dispersal (Kidd, 2018). Some riverine systems have been left fragmented by multiple impoundments resulting in isolated freshwater mussel populations. Other issues linked to freshwater mussel decline include point-source pollution (Nedeau et al., 2000; Strayer, 2006), the establishment of invasive species (Baker & Hornbach, 1997; Strayer et al., 1999), and climate change (Daufresne et al., 2004; van Vliet et al., 2013). The use of fish hosts by freshwater mussels to nourish and disperse their young makes freshwater mussels vulnerable to impacts that affect their host as well (Vaughn, 2012; Lopes-Lima et al., 2014).

Freshwater mussels have many physical habitat requirements that can vary among species (Strayer, 2008). Species can be classified as habitat generalists or specialists based on their tolerance for different habitat conditions (Haag, 2012). Due to their lack of mobility and their relatively limited mechanisms of dispersal, freshwater mussels need suitable habitat for all life stages to ensure successful recruitment (McMahon & Bogan, 2001; Strayer, 2008). The relationship between freshwater mussels and their habitat variables has been studied by other scientists with mixed results (e.g., Layzer & Madison, 1995; Smith & Meyer, 2010; Johnson & Brown, 2011; Daniel et al., 2018). Most malacologists would agree that our understanding of the complex relationship between mussels and their habitat, and our ability to predict mussel occurrence remains uncertain (Strayer, 2008; Freshwater Mollusk Conservation Society, 2016).

Recent field surveys suggest that freshwater mussel populations in Atlantic Canada have declined dramatically over the past century (Nedeau et al., 2000; Martel et al., 2010). There is limited information about freshwater mussel populations in Atlantic Canada, and new and modern investigations and monitoring have been called for (e.g., Sabine et al., 2004; Martel et al., 2010; Wegscheider et al., 2019). Most of the surveys completed in Atlantic Canada have been casual, measuring the relative surface abundance of mussels in smaller rivers or in small portions of large rivers (e.g., Hanson & Locke, 2001; Richardson et al., 2002; Locke et al., 2003; Sabine et al., 2004;

Wegscheider et al., 2019). The challenge with data collected to date is a lack of absolute numbers of density estimates, evidence of juvenile recruitment, or links to the glochidia dispersal and their hosts. For example, casual surveys do not account for juveniles who dwell in the substrate below the surface (Hornbach & Deneka, 1996; Metcalfe-Smith et al., 2000).

My objectives were to conduct baseline assessments and establish monitoring sites for freshwater mussel populations and their habitats throughout the Wolastoq | Saint John River (W|SJR), New Brunswick, and simultaneously assess physical habitat towards an attempt to describe habitat preferences in the W|SJR. Chapter 2 provides baseline population metrics (diversity, density, and abundance) for native freshwater mussel species, as well as an update on a species-at-risk, *Lampsilis cariosa*, commonly known as Yellow Lampmussel. Chapter 3 investigates relationships between freshwater mussel metrics and physical habitat by assessing associations between population metrics and habitat variables. Chapter 4 summarizes and discusses the implications of my findings for freshwater mussel research, their conservation, and effective monitoring programs.

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# Chapter 2 - Freshwater Mussel Diversity and Abundance in the Wolastoq | Saint John River, New Brunswick, Canada


## ABSTRACT

Freshwater mussels are keystone species and ecosystem engineers that provide essential services for the freshwater environment and concurrently provide ideal bioindicators of ecosystem health. Freshwater mussels are one of the most vulnerable taxa in North America, and their populations continue to decline due to habitat degradation and destruction, mostly in the form of anthropogenic impacts such as urbanization, point source pollution, and the construction and operation of impoundments. Freshwater mussel surveys allow for status updates of sensitive or endangered species as well as the development of population metrics that can inform management and conservation decisions. I performed exploratory surveys for freshwater mussels in the Wolastoq | Saint John River, located in New Brunswick, Canada. I established baseline population metrics (i.e., freshwater mussel abundance, density, and diversity), identified potential long-term monitoring locations, and provided an update on an important species-at-risk, *Lampsilis cariosa* (Yellow Lampmussel), in a river with diverse mussel assemblages and a lack of historical and current mussel data. This baseline information will expand our knowledge of the freshwater mussel species inhabiting the Wolastoq | Saint John River and provides a foundation for further freshwater mussel research and population monitoring. These metrics can be used to help guide conservation efforts and management decisions to better protect freshwater mussel communities in the Wolastoq | Saint John River in the face of continued threats such as

increased urbanization, a potential dam removal/renovation, the potential invasion of non-native organisms, and climate change.

## INTRODUCTION

Freshwater mussels are keystone species and ecosystem engineers providing numerous benefits to freshwater ecosystems and other aquatic organisms (e.g., Gutiérrez et al., 2003; Spooner & Vaughn, 2006; Vaughn, 2010; Spooner & Vaughn, 2012; Vaughn, 2018). They are filter feeders that remove organic and inorganic materials from the water column and deposit nutrients to the sediment in the form of feces or pseudofaeces (expelled suspended particles that cannot be used as food; Strayer et al., 1999; Vaughn, 2018). This bio-filtration and bio-deposition cycle of nutrients actively connects the pelagic and benthic habitats of freshwater systems (Howard & Cuffey, 2006; Vaughn, 2018). Freshwater mussels provide other important ecosystem services such as the bioturbation of sediments (Vaughn & Hakenkamp, 2001; Zimmerman & de Szalay, 2007), stabilization and oxygenation of substrates (Nedeau, 2008; Strayer, 2008), and provide habitat for other benthic organisms like macroinvertebrates, protozoans, algae, and invasive freshwater mussels (Nedeau et al., 2000; Spooner & Vaughn, 2006).

Freshwater mussels have many attributes that make them ideal bioindicators of ecosystem health (Naimo, 1995; Angelo et al., 2009). As filter feeders with a relatively long lifespan and a sedentary lifestyle, mussels readily accumulate toxins from the water column and the surrounding sediment offering a reflection of these aspects of their environmental conditions (Naimo, 1995; Khan et al., 2018). They often make up the largest portion of living benthic biomass (up to 90-95%) in undisturbed river systems

(Parmalee & Bogan, 1998; Nedeau, 2008), and mussel biomass declines in disturbed river systems can negatively impact other organisms and ecosystem functions (Strayer et al., 1999; Vaughn et al., 2004).

Compared to other molluscs, freshwater mussels have a unique lifecycle. Freshwater mussel larvae, referred to as glochidia, are shed from a mature female mainly into the water column (Mackie, 1984; Haag & Warren, 2003). At this life stage, the glochidia require a fish host to obtain the nourishment necessary for growth and development, and for dispersal (Haag & Warren, 1997; Barnhart et al., 2008). Glochidia attach to the gills or scales of their fish host where they encyst in the host tissues and feed on the fish from within the cyst (Kat, 1984; Nedeau et al., 2000). Once developed (can be a matter of days or weeks depending on species and water temperature), the metamorphosed glochidia will drop off its host to begin its juvenile life stage spending the next few years maturing into adulthood in the interstitial spaces of the substrate (Nedeau et al., 2000; Martel. Et al., 2010). Due to the essential nature of fish as hosts in the freshwater mussel lifecycle, the distribution, diversity, and abundance of fish directly impact that of their mussel dependents (Watters, 1992; Vaughn, 2012). Threats to fish such as loss of river connectivity due to dams, water quality, and loss of habitat, have also been identified as contributors to freshwater mussel decline (Martel et al., 2010; Haag & Williams, 2014). The loss of fish host availability limits both the freshwater mussel life cycle and their dispersal mechanisms and threatens the persistence of freshwater mussel populations (Schwalb et al., 2011; Lopes-Lima et al., 2014).

Over the past decades, freshwater mussels have become one of North America's most vulnerable aquatic taxa (Haag & Williams, 2014; Collier et al., 2016). Freshwater mussels have been negatively affected by human development in the form of urbanization (Stepenuck et al., 2002; Deacon et al., 2005), point source pollution (Naimo, 1995; Strayer, 2006), impoundments (Bogan, 1993; Gangloff & Feminella, 2007), invasive species (Baker & Hornbach, 1997; Strayer et al., 1999), and climatic change due to global warming (Daufresne et al., 2007; van Vliet et al., 2013). Only one third of all North American freshwater mussel species are considered ecologically secure (Strayer & Dudgeon, 2010), and therefore, it is becoming increasingly important to quantify and monitor freshwater mussel populations and distributions.

My study takes place in the Wolastoq | Saint John River (W|SJR), a large and heavily modified river system located in New Brunswick, Canada. At 673 km long with a drainage area of 55,000 $km^2$ and a mean annual discharge of 1,100 $m^3/s$, the W|SJR is the largest river along the eastern seaboard of North America (Cunjak & Newbury, 2005). The river originates in northern Maine and discharges into the Atlantic Ocean at the Bay of Fundy in New Brunswick. The river experiences different and significant impacts along its length in the state of Maine and the provinces of Quebec and New Brunswick. The upper reach of the W|SJR is impacted by potential inputs from forest harvesting activities in the north and pulp and paper mills around Edmundston. The middle and lower reaches are modified or impacted by many anthropogenic factors including forestry operations, pulp and paper mills, municipal sewage and waste treatment, urban runoff, industrial effluents, potato cultivation, and flow regulation at major and minor hydroelectric facilities and dams (Kidd et al., 2011; Kidd, 2018).

The W|SJR has three discernable reaches: upper, middle, and lower (Cunjak & Newbury, 2005; Curry & Munkittrick, 2005). The upper reach of the W|SJR flows from the headwaters to Grand Falls (the only natural barrier to fish movement in the mainstem of the river), and the lower reach flows from the Mactaquac hydroelectric generating station (MGS) to Saint John Harbour at the Bay of Fundy. The upper reach of the W|SJR is free flowing (no flow regulation structures) with predominantly gravel and cobble substrates. The middle reach of the river has modified flows at the Grand Falls hydroelectric generating station (GGS) and Beechwood hydroelectric generating station (BGS), both of which are run-of-the-river dams with limited headpond storage. The GGS and BGS have reservoirs where flows are slow, and substrates are dominated by silt and sand due to the collection of fine sediments in the slow-moving waters. Downstream of the dams, the flows scour away fine sediments and substrates are dominated by cobbles and gravels until the flows slow farther downstream. The flow in the lower reach of the W|SJR is altered and influenced by the MGS, a large run-of-the-river dam. The reservoir above the dam extends 100 km upstream and has habitat conditions more characteristic of a lake than a riverine system. Except for the area immediately downstream of the MGS, the lower reach of the W|SJR is characterized as having slow flowing waters (outside of freshet), large islands, and extensive sand bars. This portion of the river is also strongly influenced by tides (head-of-tide is approximately 150 km upstream of the mouth) and saltwater in the lower 46 km of the river (Sabine et al., 2004; Reinhart et al., 2018).

The W|SJR supports the highest diversity of freshwater mussels in Atlantic Canada (Martel et al., 2010). Eleven species of freshwater mussels have been observed in the W|SJR basin including Eastern Elliptio [*Elliptio complanata*, (Lightfoot, 1786)], Eastern Lampmussel [*Lampsilis radiata*, (Gmelin, 1791)], Yellow Lampmussel [*Lampsilis cariosa*, (Say, 1817)], Eastern Floater [*Pyganodon cataracta*, (Say, 1817)], Triangle Floater [*Alasmidonta undulata*, (Say, 1817)], Alewife Floater [*Anodonta implicata*, (Say, 1829)], Brook Floater (*Alasmidonta varicosa*, (Lamarck, 1819)], Creeper [*Strophitus undulatus*, (Say, 1817)], Tidewater Mucket [*Leptodea ochracea*, (Say, 1817)], Eastern Pearlshell [*Margaritifera margaritifera*, (Linnaeus, 1758)], and Dwarf Wedgemussel [*Alasmidonta heterodon*, (Lea, 1830)]. Two species, *L. cariosa* and *A. varicosa,* are federally listed as of 'Special Concern' under the Species at Risk Act (SARA), and one species, *A. heterodon,* has been extirpated from the region (Committee on the Status of Endangered Wildlife in Canada, 2000; COSEWIC, 2004, COSEWIC, 2009). Some species, such as *A. implicata, A. varicosa, L. ochracea, M. margaritifera,* and *L. cariosa* are of potentially important ecological value as they are endemic to Eastern Canada and are not found in other regions of the country (Nedeau et al., 2000; Martel et al., 2010).

There is a lack of current and historical data for the freshwater mussels of the W|SJR (see: Sabine et al., 2004; Martel et al., 2010; Wegscheider et al., 2019). The need for baseline data is becoming increasingly evident with proposed modifications to the MGS and increased urbanization along the banks of the river (Kidd, 2018; Curry et al., 2020). The W|SJR is also expected to experience large environmental variation due to climate change, such as an increase in drought and flood conditions, as well as saltwater intrusion as sea levels rise (Dugdale et al., 2018; Kidd, 2018). The W|SJR is also at risk for the invasion of the zebra mussel (*Dreissena polymorpha*), an invasive species that has destroyed native freshwater mussel communities across North America, yet has not been identified in the W|SJR to date (Department of Fisheries and Oceans, 2010; Martel. et al., 2010).

This study investigates freshwater mussel distributions throughout the W|SJR to establish baseline community and population metrics of species diversity, abundance (catch per unit effort (CPUE) and density), body size, and evidence of recent recruitment (presence of juveniles). My overarching goal is to develop a baseline for freshwater mussel abundance and distribution in the W|SJR, and to support developing meaningful metrics that ensure success of ongoing or future monitoring programs for the conservation the freshwater mussel communities of the W|SJR. Given the variability of habitats and disturbances along the river, I predicted mussel diversity and abundance to be variable throughout the W|SJR with significant increases downstream of the last dam where low river gradients and fine sediment dominate the substrate (Walling & He, 1998; Wampler, 2012), fish diversity is high (DFO, 2009; Kidd et al., 2011), there are no connectivity challenges, and no apparent major disturbances (Kidd et al., 2011; Wampler, 2012). I also predict that diversity, abundance, and juvenile presence/recruitment is related to human impacts and therefore will increase with distance from the dams (MGS, BGS, and GGS), the towns of Edmundston and Fredericton, and the major agricultural areas of Florenceville and Hartland (Vaughn & Taylor, 1999; Galbraith & Vaughn, 2011).

## METHODS

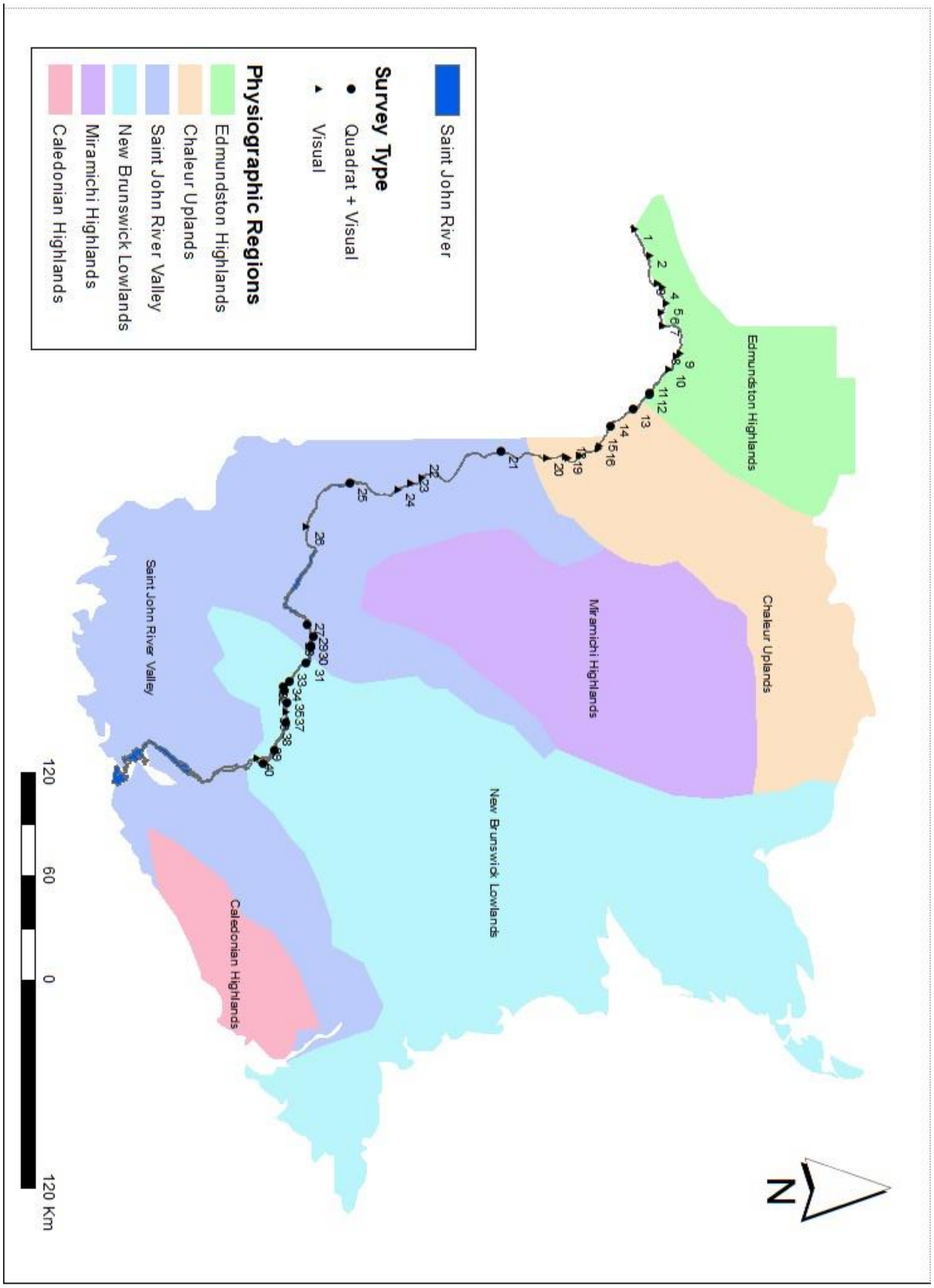
*Site Selection*

The study was conducted within four physiographic regions along the New Brunswick portion of the W|SJR: Edmundston Highlands (EH), Chaleur Uplands (CU), Saint John River Valley (SJRV), and New Brunswick Lowlands (NBL; Figure 2.1). Physiographic regions are classified based on changes in geology and geographical features, thus creating different ecosystems for aquatic life. Sites within regions were selected based on habitat suitability and site accessibility using satellite imagery followed by reconnaissance site visits to confirm habitat suitability. Habitat suitability was based on water velocity and substrate size. Sites with visibly high velocities were excluded (in part due to safety concerns and also because high flows are not conducive to good mussel habitat), and sites with larger substrate types were avoided when possible but were not excluded if flows were low to moderate because bedrock and boulder substrates are characteristic of the upstream reaches of the W|SJR. It was not possible to access enough sites to stratify sampling among habitat types, instead I selected 10 sites randomly among the available sites within each of the four physiographic regions (Figure 2.1). At each of the 40 sites (n = 10 within each of four regions), velocity was measured at the time of sampling using the surface float method, averaging five measurements for a mean that was used to characterize the velocity at a site.  Dominant and subdominant substrates at each site were classified using a modified Wentworth scale (see Gautreau et al., 2015).

*Mussel Sampling*

A field team of two to four people completed all field sampling from June to August 2018.  Summer sampling was chosen to reduce seasonal bias because freshwater mussels display strong seasonal variation throughout the year (Schwalb & Pusch, 2007; Negishi et al., 2011).

**Figure 2. 1: Sampling locations in the Wolastoq | Saint John River with associated physiographic region. Sampling sites are identified by survey type employed at each location.**



Mussels often form dense mussel beds which can be patchily distributed and separated by areas where habitat conditions are not suitable (Strayer, 2008). If mussel beds were sparse, or mussels were found randomly dispersed on the riverbed (e.g., <1 mussel per $m^2$), a timed-visual survey was conducted. When dense mussel beds were found (e.g., >1 mussel per $m^2$ and extending over 10 m of riverbed), both a timed-visual survey and a quadrat survey were conducted. Upon arrival at a site, an initial visual assessment was used to determine the presence and abundance of mussel beds and the appropriate method(s) to employ at the site (see Table 2.1 for the sampling method(s) employed at each site). No mussels were removed from the substrate at this stage.

Timed-visual surveys measured catch-per-unit-effort (CPUE) in mussels observed per hour and were conducted at sites with low mussel abundance and an absence of mussel beds. The timed-visual surveys had an average sampling effort of 1.9 person hours (SD = 0.25 person hours, range = 0.25 to 4.5 person hours) across all sites. All mussels encountered during the visual survey were collected by hand while searching wadable habitat (water depths to a maximum of 2 m) while wading or snorkeling. Search groups were coordinated so that each individual searcher was covering a separate area with little to no overlap with the other members of the search group. After the timed-visual search, mussels were identified and enumerated. All mussels were returned to the location from which they were collected.

Quadrat sampling measured mussel densities in mussels/$m^2$ and was conducted at sites with high abundance or dense mussel beds. A 50 $m^2$ area was sectioned off around a mussel bed (typically 5 x 10 m or 2 x 25 m) and divided roughly into 1 $m^2$ sections. A random number generator was used to randomly select 10 sections to sample. A 0.25 $m^2$ quadrat-sieve device (see McAlpine & Sollows, 2014) was randomly placed in the section. Mussels visible on the surface of the substrate were removed from the quadrat, and then the quadrat was excavated to the maximum depth possible (10-15 cm) or until mussels were no longer found. Quadrat surveys generally took 1 to 2 hours depending on the abundance of mussels in the targeted bed (i.e., the more mussels present, the longer the survey). Surface (epi) and subsurface (endo) mussels were collected and processed separately. At the end of the quadrat search, all mussels were identified, enumerated, measured, and weighed. Quadrat surveys were always conducted prior to a timed-visual survey. At a site with mussel beds, a timed-visual survey was conducted for 0.5 person hours to estimate CPUE at the site (as per the timed survey, see above) after quadrat sampling. One estimate was made within the delineated quadrat survey area (to ensure that the random placement of the quadrats did not miss other individuals or diversity in the delineated area). A second estimate was made outside the delineated area such that the final CPUE for quadrat sampled areas was the combined CPUE of the estimate within the delineated quadrat area and the estimate outside of the quadrat area. After the timed-visual search, the mussels were identified and enumerated. Once both the quadrat survey and the timed-visual survey had been completed, all mussels were returned to the location from which they were collected.

*Metrics*

Diversity at a site was calculated using the Shannon-Wiener diversity index (H):

$$H = -\sum_{t=1}^{R} p_i \ln p_i$$

where *R* is the total number of species in the sample and $p_i$ is the number of individuals within species *I* divided by the total number of samples. Relative abundance was measured as catch-per-unit-effort (CPUE): mussels/hour of visual survey. Density was calculated as the number of mussels / 0.25 $m^2$ (quadrat-sieve) converted to mussels/$m^2$. Total shell length (error range of ± 0.5 mm) was manually measured using calipers and wet weight (error range of ± 0.5 g) using an OHAUS Navigator portable balance. We defined juveniles as mussels ≤ 30 mm in total length (Cosgrove & Hastie, 2001; Allen & Vaughn, 2010).

**Table 2.1: Sampling method(s) used at each site based on freshwater mussel abundance and presence/absence of mussel beds. See text for detailed description of sampling methods for timed-visual surveys and quadrat sampling.**

| Region | Site Number | Method |
|---|---|---|
| Edmundston Highlands (*n* = 10 sites) | SJR1 | Visual |
| | SJR2 | Visual |
| | SJR3 | Visual |
| | SJR4 | Visual |
| | SJR5 | Visual |
| | SJR6 | Visual |
| | SJR7 | Visual |
| | SJR8 | Visual |
| | SJR9 | Visual |
| | SJR10 | Visual |
| Chaleur Uplands (*n* =10 sites) | SJR11 | Visual + Quadrat |
| | SJR12 | Visual + Quadrat |
| | SJR13 | Visual + Quadrat |
| | SJR14 | Visual + Quadrat |
| | SJR15 | Visual |
| | SJR16 | Visual |
| | SJR17 | Visual |
| | SJR18 | Visual |

| | | |
|---|---|---|
| | SJR19 | Visual |
| | SJR20 | Visual |
| Saint John River Valley (*n* = 10 sites) | SJR21 | Visual + Quadrat |
| | SJR22 | Visual |
| | SJR23 | Visual |
| | SJR24 | Visual |
| | SJR25 | Visual + Quadrat |
| | SJR26 | Visual |
| | SJR27 | Visual + Quadrat |
| | SJR28 | Visual + Quadrat |
| | SJR29 | Visual + Quadrat |
| | SJR30 | Visual + Quadrat |
| New Brunswick Lowlands (*n* = 10 sites) | SJR31 | Visual + Quadrat |
| | SJR32 | Visual + Quadrat |
| | SJR33 | Visual + Quadrat |
| | SJR34 | Visual + Quadrat |
| | SJR35 | Visual + Quadrat |
| | SJR36 | Visual |
| | SJR37 | Visual + Quadrat |
| | SJR38 | Visual + Quadrat |
| | SJR39 | Visual + Quadrat |
| | SJR40 | Visual |

*Data Analysis*

Regional differences in freshwater mussel valve lengths for species encountered in the W|SJR were analyzed using a general linear model (one-way analysis of variance; ANOVA) and a Tukey post-hoc analysis with a significance level of 0.05. All biological data were tested for normality using a Shapiro-Wilk normality test and for homogeneity of variance using a Levene's test prior to running the ANOVA model. If the biological data violated an assumption of the ANOVA (normal distribution, equal variances) and could not be corrected with a transformation, then a non-parametric Kruskal-Wallis (H) analysis with a Dunn's post-hoc test was used. All statistical analyses were performed using R Studio Version 3.6.3 statistical software using *stats, car,* and *FSA* packages (R Core Team, 2019).

## RESULTS

### *Freshwater Mussel Collections*

Sampling occurred from 3 July to 21 August 2018 at 40 sites; 11 sites had no mussels present. We observed seven species and counted and measured 4,850 mussels (Table 2.2). The average number of species at a site was 3 (range = 1 – 6 species). The most common species was *E. complanata* representing 79% of all individuals observed. Three species dominated samples (93%) with *E. complanata* accounting for 79%, *P. cataracta* accounting for 9% and *L. radiata* accounting for 5%. *L. cariosa*, a local SARA-listed species, represented <1% of individuals observed during sampling (Figure 2.2).

**Table 2.2: Total freshwater mussel counts from the visual surveys (*n* = 10 sites per region) for the seven species encountered within each region of the Wolastoq | Saint John River (see Figure 2-1 for site locations and Table 2-1 for sampling method(s) applied at each site), July to August 2018. Where: *Ec = E. complanata, Lr = L. radiata, Pc = P. cataracta, Ai = A. implicata, Au = A. undulata, Lo = L. ochracea, Lc = L. cariosa,* and rkm is river kilometer.**

| Region | River Distance (rkm) | *Ec* | *Lr* | *Pc* | *Ai* | *Au* | *Lo* | *Lc* | Total Count |
|---|---|---|---|---|---|---|---|---|---|
| EH (*n* =10) | 2 – 76 | 391 | 0 | 2 | 0 | 18 | 0 | 0 | 411 |
| CU (*n* = 10) | 88 – 147 | 1,088 | 13 | 46 | 0 | 26 | 0 | 0 | 1,173 |
| SJRV (*n* = 10) | 168 – 336 | 695 | 114 | 58 | 66 | 0 | 20 | 0 | 953 |
| NBL (*n* = 10) | 343 – 395 | 1,663 | 119 | 333 | 174 | 4 | 19 | 1 | 2,313 |
| **Total Count** | | **3,837** | **246** | **439** | **240** | **48** | **39** | **1** | **4,850** |

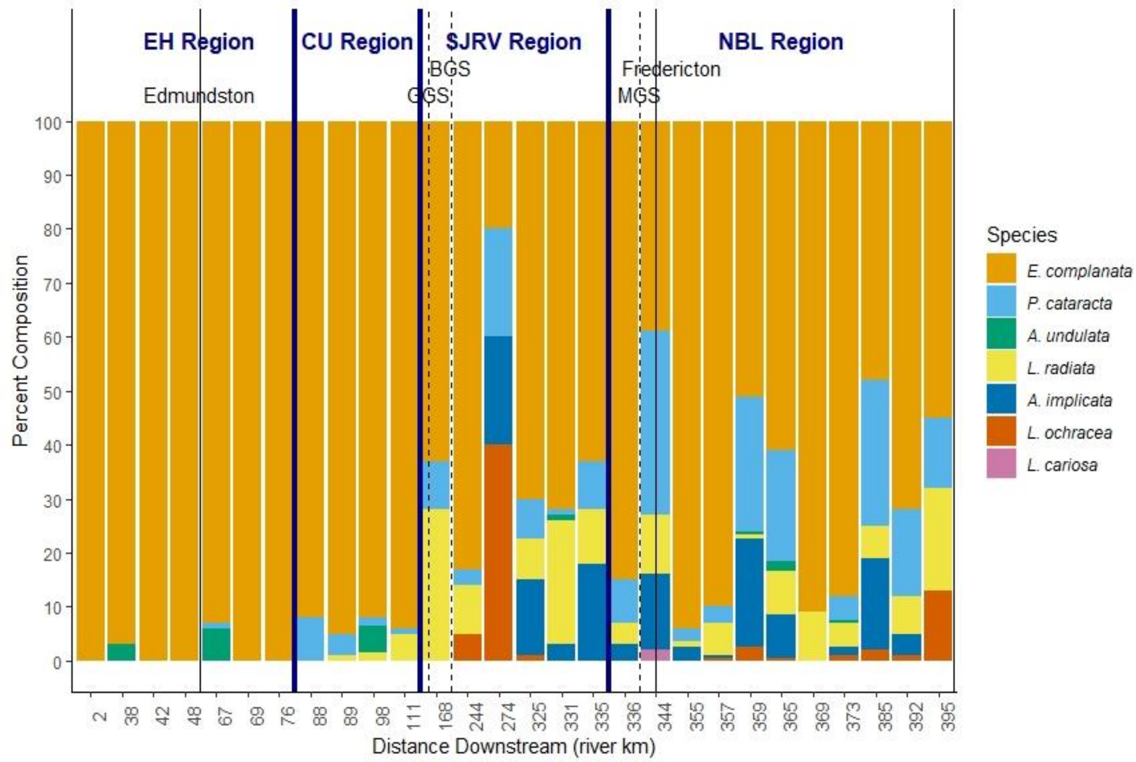



**Figure 2.1: Freshwater mussel species composition based on all samples with distance downstream from the New Brunswick/Quebec border in the Wolastoq | Saint John River, July to August 2018. Dashed lines indicate the hydroelectric generating stations (Grand Falls hydroelectric generating station [GGS], Beechwood hydroelectric generating station [BGS], and Mactaquac hydroelectric generating station [MGS]) and solid lines indicate a larger, urban centre (see Figure 2.1). See Table 2.1 for sampling method(s) used at each site.**

*Diversity and Abundance*

Freshwater mussel species richness and diversity increased with increasing distance downstream in the river. Richness averaged 2.5 mussels/site (range = 0 – 6) and diversity averaged 0.401 (range = 0 – 1.33) across the study sites. The greatest species richness and diversity was found in the farthest downstream reach (NBL region), and the lowest species richness and diversity was found in the farthest upstream reach (EH region; Table 2.3).

**Table 2.3: Freshwater mussel species richness and diversity across the regions (*n* = 10 sites per region) of the Wolastoq | Saint John River, July to August 2018 (see Figure 2.1 for site locations and Table 2.1 for sampling method(s) applied at each site).**

| | Species Richness | | SW Diversity Index (H) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Edmundston Highlands (*n* = 10) | 1.1 | 0.9 | 0.04 | 0.08 |
| Chaleur Uplands (*n* = 10) | 1.2 | 1.8 | 0.13 | 1.84 |
| Saint John River Valley (*n* =10) | 2.9 | 2.1 | 0.62 | 0.48 |
| New Brunswick Lowlands (*n* = 10) | 4.8 | 1.2 | 0.84 | 0.41 |

The CPUE measured during the visual-timed surveys averaged 191 mussels/hr (SD = 39, range = 0 – 922 mussels/hr) across sites (Table A-1). CPUE generally increased with distance downstream (Figure 2.3, Figure 2.4). *E. complanata* was the most abundant mussel found in the surveys with an average relative abundance of 146 (SD = 31, range = 0 to 810) mussels/hr per site. All species of mussel were more abundant in the downstream reaches (SJRV and NBL regions), except for *A. undulata*, which was most abundant in the CU region.

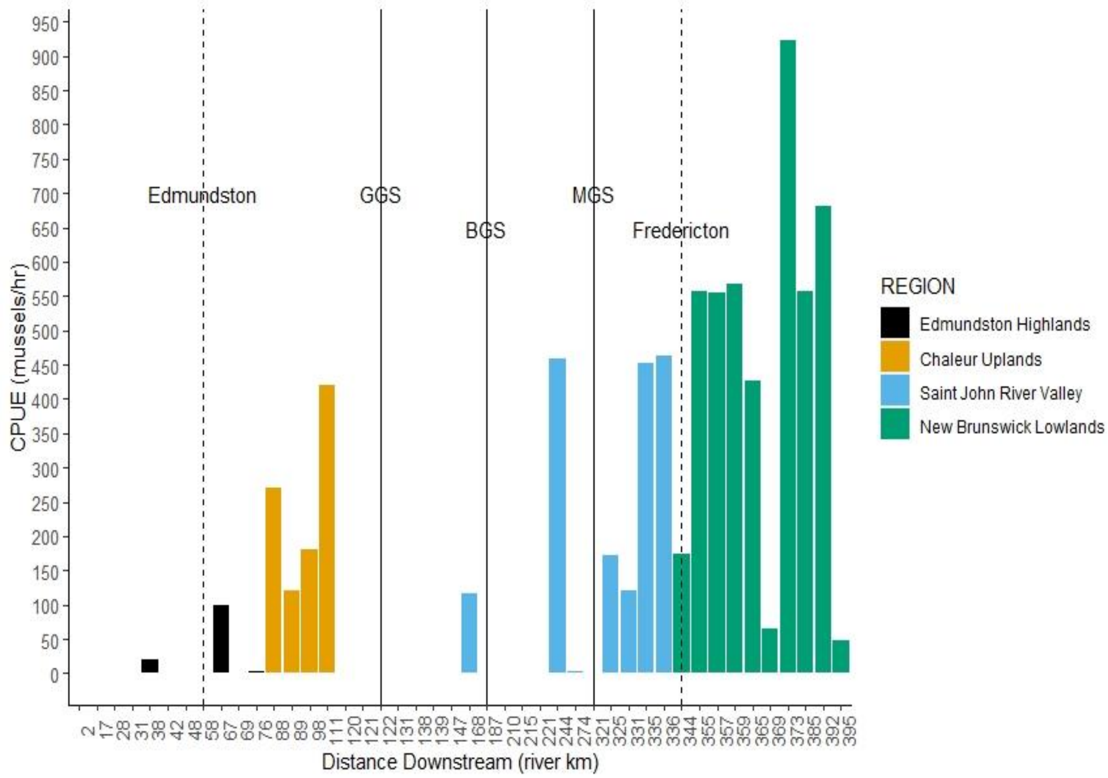



**Figure 2.2: Total catch per unit effort per site with distance downstream in the Wolastoq | Saint John River, July to August 2018. Solid lines indicate hydroelectric generating station (Grand Falls hydroelectric generating station [GGS], Beechwood hydroelectric generating station [BGS], and Mactaquac hydroelectric generating station [MGS]) and dashed lines indicate a larger urban centre.**

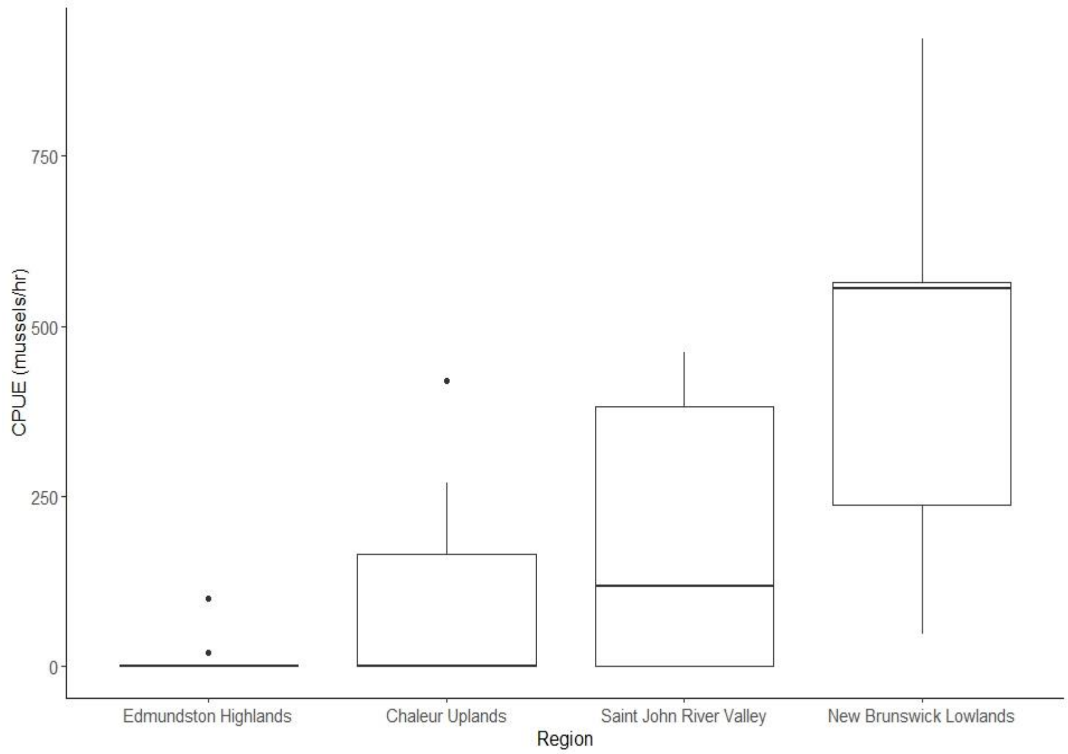



**Figure 2.3: Visual surveys as catch per unit effort, mussels/hour ± SD, across the regions of the Wolastoq | Saint John River in July to August 2018 (*n* = 10 sites for each physiographic region; see Figure 2.1).**

The mean freshwater mussel density between the regions was 8.55 mussels/$m^2$ (SD = 2.2, range = 0 – 46.4; Table A-2.). Due to low mussel abundance and the absence of mussel beds in the EH region, quadrat sampling was not employed, and density estimates were not calculated for these sites. There was a trend of increasing mussel density with increasing distance downstream (Figure 2.5). *E. complanata* was the mussel species with the greatest density found in the surveys with a mean density of 6.87 mussels/$m^2$ per site (SD = 1.9, range: 0 – 46.4).

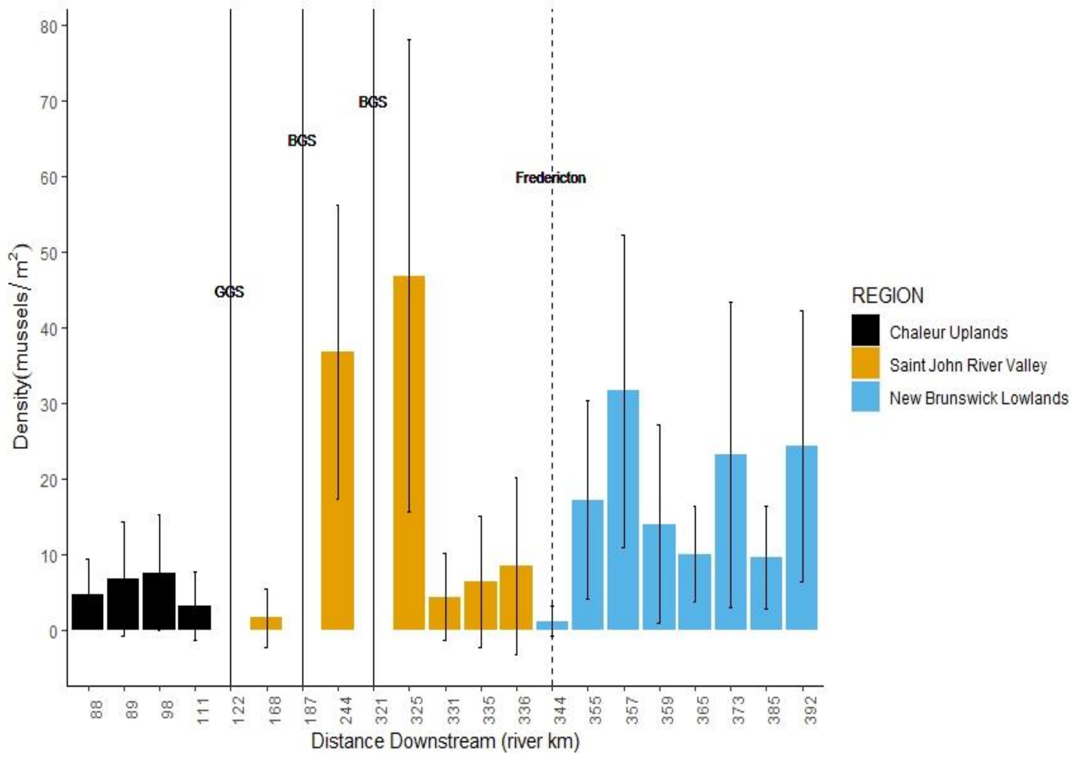



**Figure 2.4: Total density of mussels per site (average ± SD) with distance downstream in the Wolastoq | Saint John River, July to August 2018. Solid lines indicate a hydroelectric generating station (Grand Falls hydroelectric generating station [GGS], Beechwood hydroelectric generating station [BGS], and Mactaquac hydroelectric generating station [MGS]) and dashed lines indicate a larger urban centre. Note that no quadrat sampling occurred in sites with low mussel occurrence (see text for explanation).**

*E. complanata* and *A. implicata* were the largest mussels and *A. undulata* and *L. ochracea* were the smallest species based on valve length (Table 2.4). Approximately 87% of the mussels collected ranged from 41-90 mm (Table A-3). The upstream reaches (EH and CU regions) generally had larger mussels than downstream reaches (SJRV and NBL regions) (Table 2.4).

**Table 2.4: Regional differences in adult freshwater mussel valve lengths (mm) for the species encountered in the Wolastoq | Saint John River study reach, July to August 2018. Bolded *p*-values indicate statistically significant results (α = 0.05) using ANOVA or Kruskal-Wallis test, and *n* is the number of sites in the region where a species was encountered. Subscript indicates regions with significantly different valve lengths.**

| | Region | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | EH | | CU | | SJRV | | NBL | | |
| Species | *n* | $\bar{x}$ (SD) | *n* | $\bar{x}$ (SD) | *n* | $\bar{x}$ (SD) | *n* | $\bar{x}$ (SD) | *p*-value |
| *E. complanata* | 7 | $70.6_{a}$ (10.2) | 4 | $71.7_{ab}$ (7.2) | 6 | $68.1_{ab}$ (12.1) | 8 | $57.0_{ab}$ (6.0) | **0.026** |
| *L. radiata* | 0 | - | 1 | 74.0 (37.0) | 5 | 62.0 (26.4) | 8 | 61.3 (17.3) | 0.723 |
| *P. cataracta* | 1 | 71.0 (26.8) | 0 | - | 5 | 46.6 (24.0) | 6 | 48.9 (27.3) | 0.290 |
| *A. implicata* | 0 | - | 0 | - | 4 | $55.9_{a}$ (36.3) | 5 | $81.2_{b}$ (42.2) | **0.015** |
| *A. undulata* | 2 | 25.1 (24.5) | 0 | - | 0 | - | 1 | 45.0 (15.9) | 0.221 |
| *L. ochracea* | 0 | - | 0 | - | 1 | $82.5_{a}$ (33.7) | 4 | $48.0_{b}$ (24.4) | **0.035** |

Endobenthic (buried) mussels were found at every site where excavation sampling was employed, except one site, SJR21. The downstream reaches (NBL region) had the most endobenthic mussels.

Juvenile freshwater mussels were rare, and only found at sites where quadrat-based surveying and excavation were employed (Table 2.1). In total, 51 juvenile mussels were collected across all study sites. The number of juveniles found greatly increased in the downstream reaches (SJRV and NBL regions) and downstream of the MGS (Table 2.5).

**Table 2.5: Juvenile freshwater mussel counts in the Wolastoq | Saint John River, July to August 2018. Most juveniles (99%) occurred downstream of Mactaquac hydroelectric generating station (See text, *n* = 12 sites). Quadrats had a sampling area of 0.25 m$^2$ (*n* = 10 quadrats per site, total area sampled = 2.5 m$^2$), and rkm denotes river kilometer.**

| Site | Distance from MGS (rkm) | Juvenile Count | Percent Juveniles (%) |
|---|---|---|---|
| SJR27 | 3.8 | 1 | 1 |
| SJR28 | 9.4 | 1 | 9 |
| SJR29 | 12.7 | 3 | 19 |
| SJR30 | 13.5 | 2 | 10 |
| SJR31 | 20.8 | 0 | 0 |
| SJR32 | 31.7 | 1 | 2 |
| SJR33 | 34.2 | 4 | 4 |
| SJR34 | 36.1 | 9 | 23 |
| SJR35 | 41.3 | 0 | 0 |
| SJR37 | 49.5 | 7 | 12 |
| SJR38 | 62.2 | 1 | 4 |
| SJR39 | 69.0 | 20 | 33 |

*L. cariosa* (SARA-listed) was rare, with only one live individual observed (SJR31), and the only shells were collected at SJR30 and SJR31 (Table 2.6; Figure 2.1).

**Table 2.6: *L. cariosa* survey results in 2018 (see Figure 2.1 for site locations) and 2001 (see Sabine et al., 2004). *L. cariosa* was only found at sites located below the Mactaquac hydroelectric generating station (see text).**

| | 2001 | | 2018 | |
|---|---|---|---|---|
| | Live | Shells | Live | Shells |
| SJR28 | 1 | 2 | 0 | 0 |
| SJR29 | 1 | 9 | 0 | 0 |
| SJR30 | 0 | 4 | 0 | 1 |
| SJR31 | 5 | 35 | 1 | 1 |
| SJR34 | 26 | 799 | 0 | 0 |
| SJR35 | 3 | 364 | 0 | 0 |
| SJR37 | 0 | 186 | 0 | 0 |
| SJR38 | 3 | 113 | 0 | 0 |
| SJR39 | 0 | 11 | 0 | 0 |

## DISCUSSION

*Diversity and Abundance*

Mussel species richness, diversity, density, and abundance in the W|SJR all increase with distance downstream. *E. complanata*, *P. cataracta,* and *A. undulatus* were the only species found upstream of Grand Falls, and seven species occurred downstream of the MGS. This pattern of increasing diversity with downstream distance is common in rivers (Rahel & Hubert, 1991; McCabe, 2011). Similar to other rivers, upstream sites in the W|SJR included a subset of the species observed at the richer, downstream sites (Rahel & Hubert, 1991; Baselga, 2010). The W|SJR distributions are also consistent with other general findings that downstream sections of fluvial systems are larger, accumulate sediments from upstream, provide more heterogeneous habitat, and thus can support a greater number of species (Connor & McCoy, 1979; Rahel & Hubert, 1991). Larger, more stable riverine environments in general promote greater species richness (Rahel & Hubert, 1991; Daniel & Brown, 2013).

The observed mussel diversity and abundance in the W|SJR is also a function of habitat conditions and availability. For example, both *L. ochracea* and *A. implicata* would not be expected in the upstream reaches of the W|SJR to at least Grand Falls because (1) *L. ochracea* appears to be limited to slow-moving, coastal lakes and rivers, and (2) *A. implicata* is limited by the distribution of its anadromous fish hosts (Alewife [*Alosa pseudoharengus*], Blueback Herring [*Alosa aestivalis*] and likely American Shad [*Alosa sapidissima*]) which would be unable to migrate upstream of a large barrier such as Grand Falls (Nedeau et al., 2000; Nislow et al., 2011). The upstream limit of both *L.*

*ochracea* and *A. implicata* was the BGS (no individuals of these species were found upstream of the dam) where habitat conditions become unsuitable for *L. ochracea* and few anadromous fish host species reach for *A. implicata.*

Density estimates using quadrat surveys were only employed at sites where moderate to high densities were observed, and effectively characterized mussel beds found at a site or region. Mussel beds were not encountered in the EH region, so density estimates were not made in this region. Mussel densities increased (along with abundance and diversity) below the MGS where the presence of suitable habitat conditions (low gradient, fine sediments, low flows) allowed for the establishment of mussel beds.

*L. cariosa* is a SARA species typically found in inland rivers and lakes dominated by fine sediments. They thrive on shallow sand bars and pure sand substrates which are abundant downstream of the MGS (Sabine et al., 2004). Suitable habitat conditions consisting of fine sediments are available both downstream of the MGS where the river is large and slow-moving and upstream of the MGS in the reservoir. However, *L. cariosa* has not been observed during similar visual surveys in the reservoir, despite the presence of suitable habitat and their proposed fish host, Yellow Perch (*Perca flavescens*) and White Perch (*Morone americana*; COSEWIC, 2004; Sabine et al., 2004; Kneeland and Rhymer, 2008).

There were differences in freshwater mussel diversities and abundances among the different physiographic regions in the W|SJR. The most upstream reach (EH region), had the lowest species diversity, likely owing to its more northern latitudinal position, its location upstream of a large natural barrier to fish migration, Grand Falls, and substrates dominated by cobble and gravel. The greatest species diversity occurred in the downstream reach (NBL region), where the river is widest, the gradient is lowest, and the substrates consisted of primarily sand and silt.

*E. complanata* was dominant throughout the W|SJR, which is common for many Atlantic rivers (Nedeau et al., 2000; Haag, 2012). The species occurred at 29 sites and the average density when observed was > 11 mussels/$m^2$. It is uncommon among Atlantic rivers for a species abundance to exceed 5 mussels/$m^2$ (Vaughn, 1997), however this number might not be comparable with what was found in this study since areas of high abundance were targeted. The abundance of *E. complanata* across the region may be due to the presence of dispersal barriers that prevented colonization of other mussel species during glacial retreat (Haag, 2012). In general, *E. complanata* is tolerant of many different habitats, it is a fish host generalist, and has plastic life history traits, and thus enables the species to be successful in a variety of habitats (Haag, 2012).

*P. cataracta* was observed in all reaches of the W|SJR. It was found at 21 sites and the average density when observed was 2 mussels/$m^2$. It is found in variety of aquatic systems (streams to lakes), preferring slow-flowing waters with fine to silty substrates where its inflated valves allow it to remain at the surface of the sediment (Nedeau et al., 2000; Martel et al., 2010).

*L. radiata* was observed upstream of Grand Falls but was not observed in the farthest upstream reach (EH region). It was found at 19 sites and the mean density when observed was 1 mussel/$m^2$. *L. radiata* can use a variety of fish hosts such as Yellow

Perch, Smallmouth Bass (*Micropterus dolomieu*), and Pumpkinseed Sunfish (*Lepomis gibbosus*) and is a habitat generalist (Nedeau et al., 2000; Martel et al., 2010).

*A. implicata* was found in the lower reaches of the W|SJR (SJRV and NBL regions) and was observed both upstream and downstream of the MGS with higher abundances downstream. It was found at 13 sites and the average density when observed was 1 mussel/$m^2$. *A. implicata* depends on anadromous fish hosts such as Alewife and Blueback Herring which are both passed upstream of the MGS. However, low numbers of Alewife and Blueback Herring make it upstream of the BGS.

Some freshwater mussel species reported to be native to New Brunswick (Nedeau et al., 2000; Martel et al., 2010) were absent from the surveys, i.e., *Strophitus undulatus, Alasmidonta varicosa, Alasmidonta heterodon,* and *Margaritifera margaritifera. S. undulatus* and *A. heterdon* are warm-water species with habitat and fish host preferences well-suited for the W|SJR. *S. undulatus,* though abundant outside of the Maritimes, is considered rare in New Brunswick, with only few confirmed specimens observed in smaller, coastal rivers (Martel et al., 2010; New Brunswick Museum, 2020). Populations of *A. heterodon* had only been observed in the Petitcodiac River and were no longer observed following the construction of the Petitcodiac River Causeway in 1968 (COSEWIC, 2000; Hanson and Locke, 2000; Locke et al., 2003). Prior to the construction of the MGS on the W|SJR, habitat conditions (slow to moderate flow conditions and a range of substrate types) may have been suitable for *A. heterodon* and its hosts, American Shad and Atlantic Salmon, (*Salmo salar*) to thrive. American Shad are currently found and are abundant in the lower reach of the W|SJR, however, populations of Atlantic Salmon have declined dramatically in this river over the past decades (DFO, 2009; Kidd et al., 2011).

*A. varicosa* and *M. margaritifera* are typically found in the middle and downstream reaches of rivers with moderate to fast flow conditions where the two species are commonly found in the fastest, riffle habitat (Athearn & Clarke, 1962). Both *A. varicosa* and *M. margaritifera* are also associated with rivers and habitats that support, or historically supported, Atlantic Salmon populations (Nedeau et al., 2000). Atlantic salmon was once abundant in the W|SJR occurring upstream to Grand Falls (Kidd et al., 2011). Much of the middle reaches of the W|SJR are now impacted by the dams and their reservoirs and intensive agriculture (Kidd et al., 2011) that introduces fine sediments to the river and alter substrate habitats (Smedley et al., 2011). *M. margaritifera* has been observed in the lower W|SJR in Hampstead (NBM, 2020), and it is likely that it was once abundant in upstream reaches when Atlantic Salmon were abundant in these areas. *A. varicosa* has not been observed in the W|SJR to date, however it is possible that *A. varicosa* inhabited the W|SJR prior to agriculture and the construction of impoundments, both of which would have deteriorated the quality of the habitat for the persistence of *A. varicosa* and Atlantic Salmon (Kidd et al., 2011).

*E. complanata*, *A. implicata* and *L. ochracea* had significant differences in average mussel length between the physiographic regions. The *E. complanata* individuals found in the NBL region were significantly smaller than those found in the EH and CU regions. *A. implicata* individuals found in the NBL region were significantly bigger than those found in the SJRV region. Sample size was small for *A. implicata*, but individuals from NBL may have been larger due to the availability of consistent, ideal habitat conditions (slower flows, and finer substrates). *L. ochracea* individuals found in the NBL region were significantly smaller than those found in the SJRV region. Sample size was also small for *L. ochracea,* but the larger size in the SJRV region may indicate preferential habitat in this area.

Excavation surveys were employed in the middle and downstream reaches to evaluate the presence and abundance of endobenthic mussels. Endobenthic mussels were found in all regions where excavation surveys were employed (CU, SJRV, and NBL) and the highest proportion of endobenthic mussels was found in the downstream reach (NBL region). At some sites, endobenthic mussels were >50% of the mussels found. These findings indicate that fine sediment habitat is important for mussels in the W|SJR and that buried mussels can make up a significant portion of the freshwater mussels at a site. This suggests that a full and complete freshwater mussel survey method needs to include an excavation survey to get an accurate and unbiased estimate of mussel abundance.

Few juvenile mussels were encountered upstream of the MGS. The lower gradient and slower flows downstream of the MGS provide more consistent habitat and fine substrates (silt and sand) that are more conducive to juvenile recruitment and settlement (Strayer, 2008). The number of juvenile mussels was low (less than 1% of the total individuals captured) closest to MGS at SJR28 (3.8 rkm from the dam). Water levels in the W|SJR fluctuate daily to meet regional energy requirements (approximately ± 1 m in water level change daily). Freshwater mussels close to the MGS may spend more time burrowed in the substrate to avoid stranding due to low flows (Schwalb &

Pusch, 2007) or dislodgement due to high flows (Di Maio & Corkum, 1995; Schwalb & Pusch, 2007). Dewatered conditions can have deleterious effects on juvenile mussels by limiting their ability to burrow and produce byssal threads that anchor juveniles in place (Archambault et al., 2013). Mussels that continuously use energy to survive in environments with frequent disturbances may have less reproductive success, and water temperature variations resulting from dam releases can inhibit reproduction (Heinricher & Layzer, 1999; Galbraith & Vaughn, 2011). Juvenile survival and successful settlement may also be reduced because of the hydropeaking nature and dramatic daily changes in flow regime at these sites closest to the MGS (Layzer et al., 1993; Strayer, 2008). The presence and sometimes considerable proportions of juveniles at many sites further downstream of the MGS show active recruitment of those mussel species (*E. complanata, L. radiata, P. cataracta*).

The occurrence of *L. cariosa,* a "species of special concern" protected by SARA, was reported in the lower W|SJR in 2001 by Sabine et al. (2004). With all American *L. cariosa* populations considered threatened (COSEWIC, 2004) and only two known populations in Canada (W|SJR system in New Brunswick and the Sydney River system in Nova Scotia), COSEWIC and the International Union for the Conservation of Nature and Natural Resources (IUCN) have suggested that the W|SJR population could be a stronghold for *L. cariosa* (COSEWIC, 2004; Bogan & Woolnough, 2017). A total of 39 *L. cariosa* specimens and over 1,500 shells were found in the W|SJR in 2001. Nine of the previously surveyed sites were surveyed in 2018, but only one live specimen and two shells were collected. Sabine et al. (2004) assumed the presence of *L. cariosa* at the lower W|SJR sites and the extensive availability of preferential sand habitat inferred that the lower W|SJR supported a large population of *L. cariosa*. *L. cariosa* is not typically found in high abundance where it occurs. It may be more prevalent in the W|SJR due to the abundance of optimal sandbar in habitat in the lower reach (Sabine et al., 2004; COSEWIC 2004) and our surveys may not have captured the species at our sampling locations. The W|SJR population of *L. cariosa* could be the largest subpopulation of the species left in the world (COSEWIC, 2004; Bogan & Woolnough, 2017), and more work is necessary to collect accurate distribution and population estimates, to protect the remaining individuals, and to determine what factors are contributing to their decline.

*Conclusion*

The goal of this study was to provide a baseline assessment of freshwater mussel distribution, diversity, abundance and to better identify future steps in the conservation of the freshwater mussel fauna in the W|SJR. The freshwater mussel assemblages of the W|SJR were similar to larger rivers along the Atlantic seaboard. The highest diversity and abundance of freshwater mussels in the mainstem occurred in the downstream reaches and downstream of the MGS where there is a lack of dispersal barriers, lower gradient habitats, finer substrates, and a greater diversity of fish species to act as fish hosts. Recommended future freshwater mussel work would include the exploration of additional habitats because the W|SJR is large and composed of a wide range of habitat types including deeper water habitats with depths > 2 m and faster flowing habitat in the headwaters (located in the state of Maine), as well as *L. cariosa*-specific surveys designed for the detection of rare species.

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## APPENDIX

Table A-1: Freshwater mussel catch per unit effort (mussels/hr) for all sites and species, with regional averages (±1 SD; *n* = 10 sites per region) and site totals in the Wolastoq | Saint John River, July to August, 2018 (see Figure 2-1 for site locations and Table 2-1 for sampling methods). Where: *Ec = E. complanata, Lr = L. radiata, Pc = P. cataracta, Ai = A. implicata, Au = A. undulata, Lo = L. ochracea, Lc = L. cariosa.*

| | *Ec* | *Lr* | *Pc* | *Ai* | *Au* | *Lo* | *Lc* | Total |
|---|---|---|---|---|---|---|---|---|
| SJR1 | 0.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0.9 |
| SJR2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR3 | 0.2 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 |
| SJR4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR5 | 20.0 | 0 | 0 | 0 | 0.4 | 0 | 0 | 20.4 |
| SJR6 | 0.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4 |
| SJR7 | 0.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4 |
| SJR8 | 93.3 | 0 | 0.7 | 0 | 5.3 | 0 | 0 | 99.3 |
| SJR9 | 0.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4 |
| SJR10 | 2.2 | 0 | 0 | 0 | 0 | 0 | 0 | 2.2 |
| **EH Region Average (SD)** | **11.8 (29.3)** | **0** | **0.1 (0.2)** | **0** | **0.6 (1.7)** | **0** | **0** | **12.4 (31.2)** |
| SJR11 | 304.0 | 0 | 10.0 | 0 | 5.8 | 0 | 0 | 320.0 |
| SJR12 | 221.0 | 0.5 | 14.1 | 0 | 7.3 | 0 | 0 | 243.0 |
| SJR13 | 160.0 | 4.0 | 4.0 | 0 | 12.0 | 0 | 0 | 180.0 |
| SJR14 | 394.0 | 22.0 | 4.0 | 0 | 0 | 0 | 0 | 420.0 |
| SJR15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **CU Region Average (SD)** | **108.0 (151.2)** | **2.7 (6.9)** | **2.9 (5.0)** | **0** | **2.5 (4.3)** | **0** | **0** | **116.0 (161.6)** |
| SJR21 | 72.0 | 33.0 | 0 | 0 | 0 | 0 | 0 | 116.0 |
| SJR22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SJR25 | 370.0 | 42.0 | 12.0 | 0 | 0 | 34.0 | 0 | 458.0 |
| SJR26 | 0.7 | 0 | 0.7 | 0.7 | 0 | 1.3 | 0 | 3.3 |
| SJR27 | 94.0 | 28.0 | 6.0 | 42.0 | 0 | 2.0 | 0 | 172.0 |
| SJR28 | 94.0 | 26.0 | 0 | 0 | 0 | 0 | 0 | 120.0 |
| SJR29 | 290.0 | 48.0 | 34.0 | 80.0 | 0 | 0 | 0 | 452.0 |
| SJR30 | 396.0 | 18.0 | 40.0 | 8.0 | 0 | 0 | 0 | 462.0 |
| **SJRV Region Average (SD)** | **132.0 (158.9)** | **19.5 (18.7)** | **9.3 (15.2)** | **13.1 (26.9)** | **0** | **3.7 (10.6)** | **0** | **178.0 (201.9)** |
| SJR31 | 104.0 | 28.0 | 92.0 | 38.0 | 0 | 0 | 0 | 175.0 |
| SJR32 | 524.0 | 6.0 | 12.0 | 16.0 | 0 | 0 | 0 | 558.0 |
| SJR33 | 500.0 | 32.0 | 18.0 | 2.0 | 0 | 2.0 | 0 | 554.0 |
| SJR34 | 278.0 | 4.0 | 148.0 | 126.0 | 0 | 12.0 | 0 | 568.0 |
| SJR35 | 276.0 | 30.0 | 86.0 | 28.0 | 6.0 | 0 | 0 | 426.0 |
| SJR36 | 58.0 | 6.0 | 0 | 0 | 0 | 0 | 0 | 64.0 |
| SJR37 | 810.0 | 44.0 | 46.0 | 14.0 | 2.0 | 6.0 | 0 | 922.0 |
| SJR38 | 276.0 | 30.0 | 148.0 | 96.0 | 0 | 8.0 | 0 | 558.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SJR39 | 482.0 | 52.0 | 114.0 | 28.0 | 0 | 6.0 | 0 | 682.0 |
| SJR40 | 36.0 | 12.0 | 8.0 | 0 | 0 | 8.0 | 0 | 64.0 |
| **NBL Region Average (SD)** | **334.0 (244.9)** | **24.4 (16.8)** | **67.2 (57.9)** | **34.8 (42.8)** | **0.80 (1.9)** | **4.20 (4.4)** | **0** | **457.0 (278.6)** |

Table A-2: Density of freshwater mussels by site and species, with regional average ± 1SD and site totals in the Wolastoq | Saint John River, July to August 2018 (see Figure 2-1 for site locations and Table 2-1 for sampling methods). A density of 0 represents low-density sites where quadrat sampling was not employed (see text for explanation). Note that no quadrat sampling occurred in the Edmundston Highlands region. Where: *Ec = E. complanata, Lr = L. radiata, Pc = P. cataracta, Ai = A. implicata, Au = A. undulata, Lo = L. ochracea, Lc = L. cariosa.*

| | *Ec* | *Lr* | *Pc* | *Ai* | *Au* | *Lo* | *Lc* | Total |
|---|---|---|---|---|---|---|---|---|
| SJR11 | 4.80 | - | - | - | - | - | - | 4.80 |
| SJR12 | 6.40 | 0.40 | - | - | - | - | - | 6.80 |
| SJR13 | 7.60 | - | - | - | - | - | - | 7.60 |
| SJR14 | 3.20 | - | - | - | - | - | - | 3.20 |
| SJR15 | - | - | - | - | - | - | - | 0 |
| SJR16 | - | - | - | - | - | - | - | 0 |
| SJR17 | - | - | - | - | - | - | - | 0 |
| SJR18 | - | - | - | - | - | - | - | 0 |
| SJR19 | - | - | - | - | - | - | - | 0 |
| SJR20 | - | - | - | - | - | - | - | 0 |
| **CU Region Average (SD)** | **2.20 (3.1)** | **0.04 (0.1)** | **0** | **0** | **0** | **0** | **0** | **2.24 (3.1)** |
| SJR21 | 1.6 | - | - | - | - | - | - | 1.60 |
| SJR22 | - | - | - | - | - | - | - | 0 |
| SJR23 | - | - | - | - | - | - | - | 0 |
| SJR24 | - | - | - | - | - | - | - | 0 |
| SJR25 | 36.8 | 3.60 | 0.80 | - | - | - | - | 36.8 |
| SJR26 | - | - | - | - | - | - | - | 0 |
| SJR27 | 46.4 | 0.40 | 4.80 | 2.80 | - | 0.8 | - | 46.4 |
| SJR28 | 4.40 | 1.20 | 0.40 | 0.80 | - | - | - | 4.40 |
| SJR29 | 6.40 | 0.40 | 1.60 | 1.20 | - | - | - | 6.40 |
| SJR30 | 8.40 | 0.40 | 0.40 | 1.20 | - | - | - | 8.40 |
| **SJRV Region Average (SD)** | **10.4 (16.9)** | **0.60 (1.1)** | **0.77 (1.5)** | **0.60 (0.9)** | **0** | **0.08 (0.3)** | **0** | **10.4 (16.9)** |
| SJR31 | 0.40 | 0.40 | - | - | - | - | 0.4 | 1.20 |
| SJR32 | 16.0 | 0.40 | 0.80 | - | - | - | - | 17.2 |
| SJR33 | 27.6 | 1.60 | 1.20 | - | - | - | - | 30.4 |
| SJR34 | 10.0 | 0.40 | 2.00 | 0.80 | 0.40 | 0.40 | - | 14.0 |
| SJR35 | 3.20 | 1.60 | 2.40 | 2.0 | 0.40 | 0.40 | - | 10.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SJR36 | - | - | - | - | - | - | - | 0 |
| SJR37 | 20.8 | 0.40 | - | 0.40 | - | 1.60 | - | 23.2 |
| SJR38 | 3.20 | 1.60 | 3.60 | 0.80 | - | 0.40 | - | 9.60 |
| SJR39 | 20.0 | 1.60 | 2.40 | 0.40 | - | - | - | 24.4 |
| SJR40 | - | - | - | - | - | - | - | 0 |
| **NBL Region Average (SD)** | **10.1 (10.3)** | **0.80 (0.7)** | **1.24 (1.3)** | **0.44 (0.6)** | **0.08 (0.2)** | **0.28 (0.5)** | **0.04 (0.1)** | **13.0 (10.8)** |

Table A-3: Freshwater mussel valve length (mm) among species of in the Wolastoq | Saint John River, July to August 2018 (see Figure 2-1 for sampling locations and Table 2-1 for sampling methods). Where: *Ec = E. complanata, Lr = L. radiata, Pc = P. cataracta, Ai = A. implicata, Au = A. undulata, Lo = L. ochracea, Lc = L. cariosa.*

| | Length Class (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | 0-10 | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | 71-80 | 81-90 | 90+ |
| *Ec* (*n* = 907) | 2 (0.2%) | 3 (0.3%) | 34 (4%) | 35 (4%) | 72 (8%) | 163 (18%) | 208 (23%) | 218 (24%) | 138 (15%) | 34 (4%) |
| *Lr* (*n* = 37) | - | - | 3 (8%) | 3 (8%) | 7 (19%) | 8 (22%) | 6 (16%) | 6 (16%) | 3 (8%) | 1 (3%) |
| *Pc* (*n* = 52) | - | 1 (2%) | 3 (5%) | 6 (12%) | 12 (23%) | 12 (23%) | 9 (17%) | 6 (12%) | 2 (4%) | 1 (2%) |
| *Ai* (*n* = 25) | - | - | - | - | - | 3 (12%) | 6 (24%) | 7 (28%) | 6 (24%) | 3 (12%) |
| *Au* (*n = 21)* | - | - | 2 (9%) | - | 12 (57%) | 7 (34%) | - | - | - | - |
| *Lo* (*n* = 9) | - | 3 (33%) | - | 1 (11%) | 2 (23%) | 1 (11%) | - | 1 (11%) | 1 (11%) | - |

# Chapter 3 - Freshwater Mussel Assemblages in Relation to Habitat Characteristics in the Wolastoq | Saint John River, New Brunswick, Canada


## ABSTRACT

Freshwater mussel populations have experienced declines over the past decades from habitat alterations caused by anthropogenic activities such as the construction of dams, point-source pollution, the introduction of invasive species, and climate change. I examined physical habitat characteristics in association with community structure in the Wolastoq | Saint John River (W|SJR), New Brunswick. I found that compositionally the W|SJR can be split into two distinct areas (northern and southern) where similar species compositions occur. Freshwater mussel density was not significantly different between regions suggesting that mussel bed densities are similar throughout the river. The NBL region had significantly higher freshwater mussel abundance and diversity compared to the other regions. Distribution and abundance of freshwater mussels in the W|SJR can largely be explained by the proximity to a dam, local geology, and barriers to dispersal. Site-level habitat variables measured at each site were determined to be poor predictors of freshwater mussel occurrence in the W|SJR.

# INTRODUCTION

Freshwater mussels are disproportionately affected by habitat change and disturbances relative to other freshwater organisms, largely because mussels are long-lived and have relatively low mobility as juveniles and adults (Grabarkiewicz & Davis, 2008; Haag & Williams, 2014). Freshwater mussel populations have declined in recent decades due to anthropogenic activities and impacts on their freshwater habitats (e.g., Bogan, 1993; Williams et al., 1993; Bogan, 2008; Collier et al., 2016). The construction of dams, overfishing, industrial and sewage effluent, are among many human activities to impact the physical, chemical, and biological characteristics of freshwater habitats for freshwater mussels (Strayer, 2006; Collier et al., 2016).

The most significant cause of freshwater mussel imperilment is habitat destruction, degradation, and modification due to human activities (e.g., Dudgeon et al., 2006; Bogan, 2008; Strayer 2008, Martel et al., 2010; Collier et al., 2016). The construction of impoundments in rivers has resulted in habitat alteration, and fragmentation and loss while also impacting flow conditions (Fuller et al., 2015; Poff & Zimmerman, 2010). The river reach below dams and impoundments may have unnatural flow, temperature, and sediment regimes that negatively impact native mussel species (Vaughn & Taylor, 1999; Gangloff et al., 2011). Freshwater mussel species and their populations, as well as those of their fish host, can be isolated below and above an impoundment (Allen et al., 2013; Randklev et al., 2016). Any change to the environment or habitat that affects the distribution of host fishes will have a direct effect on the distribution of freshwater mussels (Nedeau et al., 2000; Martel et al., 2010). Other human modifications that impact riverine systems and indirectly affect freshwater mussel assemblages include channelization, the destruction of wetlands and shallow-water habitats, and the removal of riparian vegetation (e.g., Brinson & Malvarez, 2002; Poole & Downing, 2004; Martel et al., 2010).

Anthropogenic pollution has also influenced the physical and chemical makeup of freshwater habitats (Alrumman et al., 2016; Amoatey & Baawain, 2019). Point source pollution by contaminants, nutrients, and organic matter has been widespread in freshwater systems around the world and have degraded freshwater habitat for freshwater mussel species (Dudgeon et al., 2006; Bogan, 2008). In areas where point source pollution is regulated, the effects of earlier contamination can be long lasting on freshwater mussel communities due to the persistence of some pollutants (e.g., metals, organics) and the relatively long lifespan of some freshwater mussel species (Strayer, 2006; Pinkney et al., 2015). Non-point source pollution, such as sediment and nutrient loading from agricultural and urban areas, also contribute to freshwater contamination. These pollutants often change physical habitat characteristics such as water chemistry, substrate composition, and depth and flow conditions. Both point source and non-point source contamination can impact freshwater mussels by interrupting physiological processes such as metabolic, respiratory, and reproductive rates, reducing growth rates, and other symptoms that may result in death of the mussel (Hinck et al., 2011; Gillis et al., 2017). Certain mussel life stages are more susceptible to pollution than others, (i.e., a pollutant that is innocuous to an adult mussel may be toxic to the glochidia or juvenile stage; Nedeau et al., 2000).

The introduction of non-native, aquatic species can significantly alter physical, chemical, and biological characteristics of lakes and rivers as well as mussel communities

(Strayer et al., 1999; Martel et al., 2010). Non-native mussels (zebra mussels [*Dreissena polymorpha*] and quagga mussels [*Dreissena bugensis*]) colonize the shells of native freshwater mussel species (often in large numbers) and effectively impair feeding, movement, and reproduction of native species (Gillis and Mackie, 1994; Martel et al., 2001). Zebra and quagga mussels use different reproductive strategies than native freshwater mussel species. They do not require internal fertilization, can release up to one million eggs in a breeding season, and the larval phase is free swimming, all of which allow the rapid invasion of non-native mussels in introduced systems (Ram et al., 1996).

Global climate change has brought, and will continue to bring, changes in weather patterns (temperature and precipitation), an increase in extreme weather events like droughts and floods, as well as rising sea levels (Pinkney et al., 2015; Collier et al., 2016). Increases in water temperature and decreases in dissolved oxygen content due to a rise in global temperatures can cause thermal stress which adversely affects the survival and physiology of freshwater mussel species and their fish hosts (Hastie et al., 2003; Ganser et al., 2013). Rising sea levels will result in the decrease of the downstream limits of many freshwater mussel species inhabiting coastal rivers due to upstream saltwater intrusion (Hastie et al., 2003).

To support healthy freshwater mussel communities, mussels require specific habitat requirements to be fulfilled (for a detailed review see Strayer, 2008). The substrate must be soft enough to promote the burrowing of both adult and juvenile mussels (Schwalb & Pusch, 2007; Archambault et al., 2013), but also, be stable enough to prevent wash-out or sediment fill-in after flooding or high flow events (Strayer & Ralley, 1993; Mcrae et al., 2004). Adult mussels require food to be available in the water column, and juveniles need adequate nourishment within the interstitial spaces of the sediment (McMahon & Bogan, 2001; Raikow & Hamilton, 2001). All life stages need access to essential elements, such as calcium and oxygen (Hincks & Mackie, 1997; Swartz & Nedeau, 2007). The surrounding environmental variables (e.g., water velocity, water temperature, etc.) need to provide and maintain conditions for growth, reproduction, and survival (Strayer, 2008; Galbraith & Vaughn, 2011). Freshwater mussels of all life stages need protection from predators and an environment that is minimally impacted by contaminants that can disrupt the freshwater mussel lifecycle and that of their fish hosts (Committee on the Status of Endangered Wildlife in Canada, 2013; Collier et al., 2016).

With many freshwater mussel populations facing imperilment around the globe (see: Bogan, 1993; Williams et al., 1993; Collier et al., 2016), it is becoming increasingly important to understand mussel-habitat relationships. Establishing baseline population and habitat preferences allows for the assessment of conditions pre-, post-, and during projects that have the potential to produce short-term and long-lasting environmental effects and the implementation of monitoring programs for sensitive habitats and at-risk populations. Developing monitoring locations with ideal environmental conditions can be useful in relocating mussel populations from at-risk or impacted areas.

This baseline study aims to provide preliminary information regarding relationships between freshwater mussels and their physical habitat in the Wolastoq | Saint John River (W|SJR) in New Brunswick, Canada. The goal is to describe associations between mussel community and populations with habitat characteristics locally (substrate type and embeddedness, water quality, and flow velocity) and regionally (physiographic setting and proximity to dams and their impoundments). Since freshwater mussels have many lifestyle requirements dependent on their physical habitat (Strayer, 2008; Haag, 2012), I predicted that there would be significant relationships between population metrics (abundance, diversity, species richness) and local or site-specific habitat characteristics. I expected embeddedness to be important in controlling mussel presence because it is typically a good representation of substrate type, stability, and suitability for burrowing (Obeysekara et al., 2009). Regionally, I predicted that I would see lower density and diversity in the northern regions of the W|SJR due to a significant dispersal barrier (Grand Falls) and larger substrate types (lower embeddedness). I expected higher measures of abundance, diversity, and mussel size in the W|SJR downstream of the lowermost dam where the gradient of the river is low and fine sediment dominate the substrate (Walling & He, 1998; Wampler, 2012). Below the dam, slower flows and smaller substrates create ideal conditions for the majority of freshwater mussel species found in the W|SJR (Nedeau et al., 2000; Martel et al., 2010), fish diversity is high (Curry & Munkittrick, 2005; Kidd et al., 2011), and there are no apparent connectivity challenges downstream (Kidd et al., 2011; Wampler, 2012).

## METHODS

*Study Area*

The W|SJR is a large and modified river system located in New Brunswick, Canada. At 673 km long, a drainage area of > 55,000 km$^2$, and a mean annual discharge of 1,100 m$^3$/s, the W|SJR is the largest river in Atlantic Canada after the St. Lawrence River (Cunjak & Newbury, 2005). The river originates in northern Maine, flows through the provinces of Quebec and New Brunswick, and discharges into the Atlantic Ocean at the Bay of Fundy in New Brunswick. Along its length, the river is modified or impacted by many anthropogenic factors including forestry operations, pulp and paper mills, municipal sewage and waste treatment, urban runoff, industrial effluents, potato cultivation, and substantial flow regulation at three hydroelectric facilities (Kidd et al., 2011; Kidd, 2018).

The W|SJR has three discernable reaches: upper, middle, and lower (Cunjak & Newbury, 2005; Curry & Munkittrick, 2005). The upper reach of the W|SJR flows from the headwaters to Grand Falls and is the only natural barrier to fish movement in the mainstem of the river. It is free flowing (no flow regulation structures) with predominantly gravel and cobble substrates. The middle reach from Grand Falls to the MGS has modified flows at Grand Falls which also has a hydroelectric generating station (GGS) and Beechwood hydroelectric generating station (BGS), both of which are run-of-the-river dams. The GGS and the BGS have impoundments where flows are slow, and substrates are dominated by silt and sand due to the collection of fine sediments. Downstream of the dams, the flows are artificially regulated but also have high velocity zones owing to the elevation drops. Substrates are dominated by cobbles and gravels close to the dams. The lower reach of the W|SJR flows from the MGS to the Bay of Fundy. The MGS, a large run-of-the-river dam, has regulated flows and coarser substrates close to the dam. The reservoir above the MGS extends 100 km upstream with conditions more characteristic of a lake than a river. Except for the area immediately downstream of the MGS, the downstream reach of the W|SJR is characterized by slower flowing waters, large islands, and extensive sand bars. This portion of the river is also strongly influenced by tides: saltwater enters the first 46 km of the river with the head-of-tide located ~142 km upstream of the mouth of the river (Kidd et al. 2011; Sabine et al., 2004).

*Site Selection*

The study was conducted across four physiographic regions along the New Brunswick portion of the W|SJR: Edmundston Highlands (EH), Chaleur Uplands (CU), Saint John River Valley (SJRV), and New Brunswick Lowlands (NBL; Table 3-1). Physiographic regions are classified based on changes in geology and geomorphological features, thus creating different aquatic ecosystems (Dawson, 1927; MacLeod et al., 2017). Sites within regions were selected based on apparent habitat suitability and site accessibility using satellite imagery followed by reconnaissance site visits to confirm habitat suitability. Habitat suitability was based on water velocity and substrate size characteristics. Sites with visibly high velocities were excluded in part due to safety concerns for sampling. Sites with larger substrate or bedrock were avoided when possible but were not excluded if flows were low to moderate, e.g., bedrock and boulder substrates are characteristic of the upstream reaches of the W|SJR. It was not possible to access enough sites to stratify sampling among habitat types, instead I selected 10 sites among the available sites within each of the four physiographic regions based on ease of access and likelihood of finding freshwater mussels (Figure 3.1).

**Figure 3.1: Sampling locations in the Wolastoq | Saint John River with associated physiographic region. Sampling sites are identified by survey type employed at each location.**

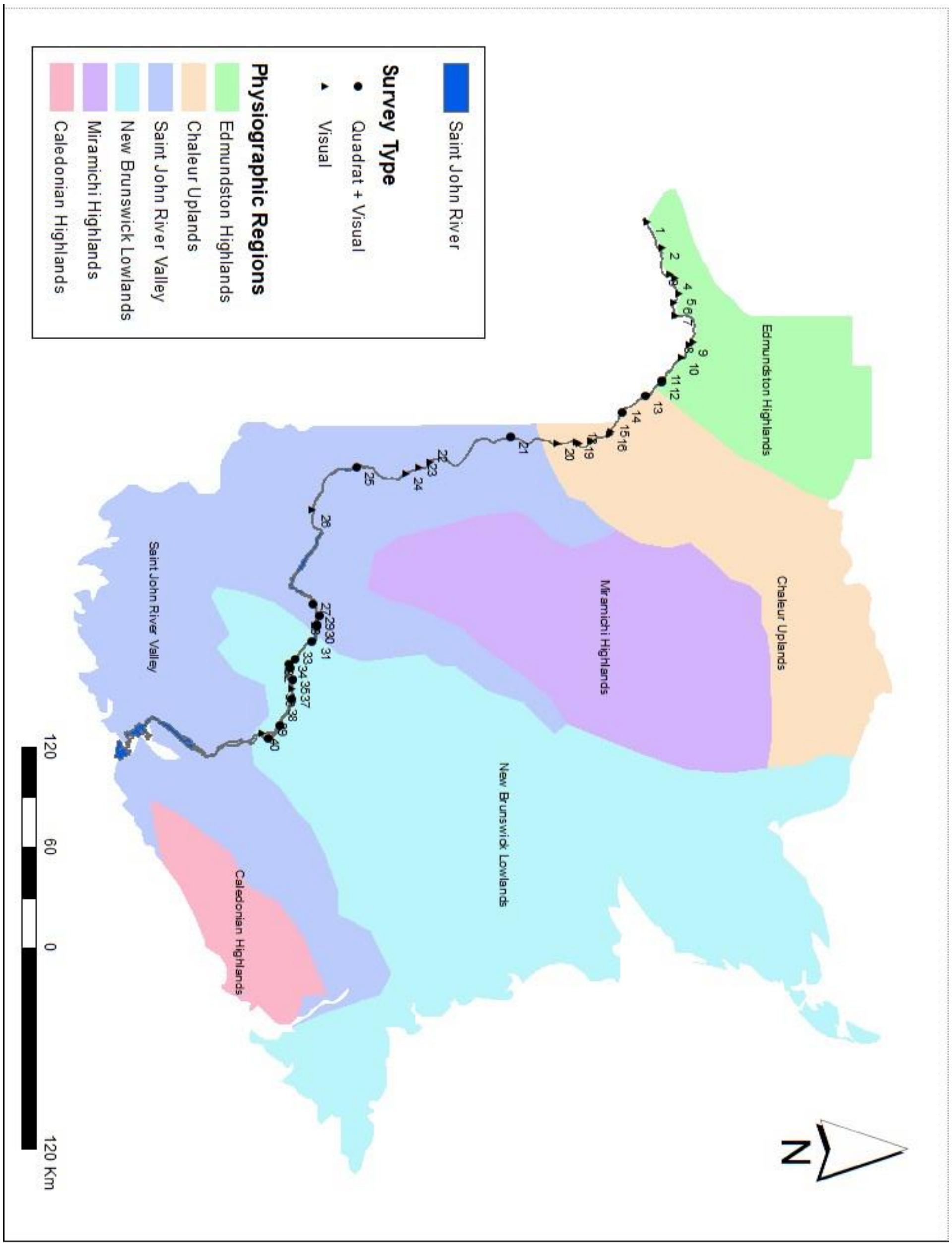

**Table 3.1: Physiographic regions of the Wolastoq | Saint John River, New Brunswick. Anthropogenic factors summarized from Curry and Munkittrick (2005).**

| Physiographic Region | River Reach | Anthropogenic Stressors |
|---|---|---|
| Edmundston Highlands (EH) | Upper | Industrial forestry, pulp and paper mills, processing and manufacturing plants, municipal waste, urban areas (Edmundston) |
| Chaleur Uplands (CU) | Middle | Industrial forestry, pulp and paper mills, potato farming and processing, municipal waste, urban areas (Grand Falls, Tobique, Aroostook), Grand Falls hydroelectric facility |
| Saint John River Valley (SJRV) | Middle | Potato farming, food processing, urban areas (Florenceville, Hartland, Woodstock, Nackawic, Kingsclear), municipal waste, Beechwood and Mactaquac hydroelectric facilities |
| New Brunswick Lowlands (NBL) | Lower | Urban areas (Fredericton, Oromocto, Gagetown, Jemseg), municipal waste, Mactaquac hydroelectric facility, mixed farming |

*Mussel Sampling*

A field team of two to four people completed all field sampling from June to August 2018. Summer sampling was chosen to reduce seasonal bias because freshwater mussels display strong seasonal variation, moving both vertically and horizontally in/on the substrate throughout the year to feed, increase reproductive success, and escape stressors such as adverse environmental conditions (Schwalb & Pusch, 2007; Negishi et al., 2011).

Mussels often form dense mussel beds which can be patchily distributed and separated by areas where habitat conditions are not suitable (Strayer, 2008). If mussel beds were sparse, or mussels were found randomly dispersed on the riverbed (e.g., <1 mussel per $m^2$), a timed-visual survey was conducted. When dense mussel beds were found (e.g., >1 mussel per $m^2$ and extending over 10 m of riverbed), both a timed-visual survey and a quadrat survey were conducted. Upon arrival at a site, an initial visual assessment (approximately 15 minutes) was used to determine the presence and abundance of mussel beds and the appropriate method(s) to employ at the site (see Table 2.1 for the sampling method(s) employed at each site). No mussels were removed from the substrate at this stage.

Timed-visual surveys measured catch-per-unit-effort (CPUE) in mussels observed per hour and were conducted at sites with low mussel abundance and an absence of mussel beds. The timed-visual surveys had an average sampling effort of 1.9 person hours (SD = 0.25 person hours, range = 0.25 to 4.5 person hours) across all sites. All mussels encountered during the visual survey were collected by hand while searching wadable habitat (water depths to a maximum of 2 m) while wading or snorkeling. Search groups were coordinated so that each individual searcher was covering a separate area with little to no overlap with the other members of the search group. After the timed-visual search, mussels were identified and enumerated. All mussels were returned to the location from which they were collected.

Quadrat sampling measured mussel densities in mussels/$m^2$ and was conducted at sites with high abundance and dense mussel beds. A 50 $m^2$ area was sectioned off around a mussel bed (typically 5 x 10 m or 2 x 25 m) and divided roughly into 1 $m^2$ sections. A random number generator was used to randomly select 10 sections to sample. A 0.25 $m^2$ quadrat-sieve device (see McAlpine & Sollows, 2014) was randomly placed in the section. Mussels visible on the surface of the substrate were removed from the quadrat, and then the quadrat was excavated to the maximum depth possible (10-15 cm) or until mussels were no longer found. Quadrat surveys generally took 1 to 2 hours depending on the abundance of mussels in the targeted bed (i.e., the more mussels present, the longer the survey). Surface (epi) and subsurface (endo) mussels were collected and processed separately. At the end of the quadrat search, all mussels were identified, enumerated, measured, and weighed. Quadrat surveys were always conducted prior to a timed-visual survey. At a site with mussel beds, a timed-visual survey was conducted for 0.5 person hours to estimate CPUE at the site (as per the timed survey, see above) after quadrat sampling. One estimate was made within the delineated quadrat survey area (to ensure that the random placement of the quadrats did not miss other individuals or diversity in the delineated area). A second estimate was made outside the delineated area such that the final CPUE for quadrat sampled areas was the combined CPUE of the estimate within the delineated quadrat area and the estimate outside of the quadrat area. After the timed-visual search, the mussels were identified and enumerated. Once both the quadrat survey and the timed-visual survey had been completed, all mussels were returned to the location from which they were collected.

*Physical Habitat Measurements*

At each of the 40 sites (n = 10 within each of four regions), temperature, electrical conductance (EC), pH, dissolved oxygen (DO), and turbidity were measured using a YSI 6 series multiparameter meter. Water quality variables were measured once at mid-channel depth at the approximate centre of the survey area. Velocity was measured using the surface float method, averaging five measurements for a mean that was used in data analyses. A correction factor was not applied to the measured surface velocities. Embeddedness at each site was determined using the visual method developed by Platts

(1982). Ten rocks/objects were randomly selected from the sampling area and given an embeddedness score of 0, 25, 50, 75, or 100%, with 0% representing rocks/objects that were fully sitting above the substrate and 100% representing rocks/objects that were fully embedded. Embeddedness is representative of the amount and type of fine sediment available to cover larger solid rocks/objects (in this case mussels; Platts, 1982) and is also a good indication of the type/size of substrate available at a site (i.e., low embeddedness would indicate large substrate type with little fine sediment present, whereas high embeddedness would indicate small substrate available to embed objects).

*Statistical Analysis*

Regional differences in freshwater mussel (CPUE), density, diversity, and mussel length were analyzed using a general linear model (analysis of variance; ANOVA) and a Student Newman Keuls (SNK) post-hoc analysis with a significance level of 0.05. If the biological data violated an assumption of the ANOVA (normal distribution, equal variances) and could not be corrected with a transformation, then a non-parametric Kruskal-Wallis (H) analysis with a Dunn's post-hoc test was used. All biological data were tested for normality using a Shapiro-Wilk normality test, as well as equal variances using a Levene's test prior to running statistical tests. Any data that did not follow a normal distribution were log-transformed (ln[x+1]).

Differences between physical habitat characteristics where mussels were present ($n$ = 29) and where mussels were absent ($n$ = 11) were examined using a Welch's $t$-test with a significance level of 0.05. All physical habitat data were tested for normality using a Shapiro-Wilk normality test prior to running statistical tests. Any data that did not follow a normal distribution were log-transformed (ln[x+1]).

Associations between the calculated population metrics and physical habitat attributes were evaluated using a Spearman rank correlation test (Table 3.2).

**Table 3.2: Population metrics and physical habitat attributes used in data analyses following a Spearman rank correlation test.**

| Population Metric | Physical Habitat Attributes |
|---|---|
| Species Diversity | Water Temperature |
| Total CPUE | pH |
| Total Density | Dissolved Oxygen |
| Species Richness | Electrical Conductance |
| Average Length | Turbidity |
| *Elliptio complanta* CPUE | Velocity |
| *Lampsilis radiata* CPUE | Embeddedness |
| *Pyganodon cataracta* CPUE | Distance from Dam |
| *Anodonta implicata* CPUE | |
| *Alasmidonta undulata* CPUE | |
| *Leptodea ochracea* CPUE | |
| *Elliptio complanata* Density | |
| *Lampsilis radiata* Density | |
| *Pyganodon cataracta* Density | |

To determine the similarity of the sampled sites among physiographic regions in terms of species abundance (CPUE) and physical habitat characteristics, non-metric multidimensional scaling (NMDS) methods using the Bray-Curtis dissimilarity distance and Euclidean distance, respectively, were used with $k$ = 2 and number of restarts = 100. The species abundance data was log-transformed.

All statistical analyses were performed using R Studio Version 3.6.3 statistical software using packages: *stats, car, FSA, Rmisc, Hmisc, PerformanceAnalytics, vegan, MASS, and ggplot2* (R Core Team, 2019).

## RESULTS

CPUE was statistically different among regions ($F_{[3, 36]} = 8.536$, $p < 0.01$; Table 3.3). The pair-wise differences occurred between NBL and the other regions ($p < 0.05$). Density was not measured in the EH region and there were no statistically significant differences among the remaining regions ($F_{[2, 27]} = 2.803$, $p > 0.05$). Diversity was statistically different among regions ($KW = 21.17$, $p < 0.01$; Table 3.3). The pair-wise differences occurred between NBL and the EH, CU regions and EH and the SJRV and NBL regions. Valve length was statistically different among regions ($F_{[3, 21]} = 3.927$, $p < 0.01$; Table 3.3). The pair-wise differences occurred between NBL and the other regions ($p < 0.05$).

A summary of regional physical habitat measurements is presented in Table 3.4. The EH region had the highest average temperatures and flows observed during the sampling period (July to August 2018). All regions were well-oxygenated and had low turbidity. Lower embeddedness values reflect sites with larger substrates, whereas higher embeddedness values reflect sites with more fine sediments available.

**Table 3.3: Regional differences in freshwater mussel CPUE, density and diversity in the Wolastoq | Saint John River study reach, July to August 2018. Values are mean with range. Bolded *p*-values indicate statistically significant results (α = 0.05) using ANOVA. Subscript indicates regions with statistically insignificant pair-wise differences.**

| | Edmundston Highlands | Chaleur Uplands | Saint John River Valley | New Brunswick Lowlands | *p*-value |
|---|---|---|---|---|---|
| CPUE (ind/hr) | 12.4 (0 – 99.3)$_a$ | 116 (0 – 420)$_a$ | 178 (0 – 462)$_a$ | 457 (64 – 922)$_b$ | **0.0002** |
| Density (no/m$^2$) | - | 2.2 (0 – 10) | 10.4 (0 – 46) | 13.0 (0 – 30) | 0.080 |
| Diversity | 0.04 (0 – 0.3)$_a$ | 0.1 (0 – 0.4)$_a$ | 0.6 (0 – 1.3)$_b$ | 0.8 (0.3 – 1.3)$_b$ | **0.00009** |
| Valve Length (mm) | 70.6 (55 – 84)$_a$ | 71.8 (62 – 77)$_a$ | 68.1 (59 – 90)$_a$ | 56.6 (49 – 70)$_b$ | **0.023** |

**Table 3.4: Summary of regional physical habitat characteristics. Values are mean with range (*n* = 10 sites/region).**

| | Edmundston Highlands | Chaleur Uplands | Saint John River Valley | New Brunswick Lowlands |
|---|---|---|---|---|
| Temperature (°C) | **25.6** (16.0 –29.2) | **22.6** (21.0 – 26.0) | **22.0** (16.8 – 25.3) | **24.9** (23.0 – 28.2) |
| pH | **7.6** (7.1 – 8.8) | **8.4** (7.1 – 9.6) | **8.0** (6.8 – 9.2) | **6.7** (5.7 – 7.9) |
| Dissolved Oxygen (mg/L) | **8.8** (8.0 – 10.7) | **9.7** (6.2 – 18.1) | **8.9** (7.3 – 11.0 | **8.1** (0.4 – 11.6) |
| Conductivity (µS/cm) | **88.9** (2 – 126) | **104** (12.4 – 140) | **138.3** (117 – 197) | **115.6** (58 – 137) |
| Turbidity (NTU) | **2.93** (0.6 – 7.1) | **3.9** (1.9 – 7.2) | **5.1** (1.4 – 19.0) | **2.4** (0.9 – 4.1) |
| Velocity (m/s) | **0.17** (0.03 – 0.50) | **0.09** (0 – 0.29) | **0.10** (0 – 0.34) | **0.047** (0 – 0.14) |
| Embeddedness | **16.8** (0 – 52.5) | **35.1** (0 – 50) | **34.0** (0 – 90) | **44.8** (0 – 50) |

Based on the results of *t*-tests which tested for significant differences in measured site-specific physical habitat characteristics between sites where mussels were present and sites without where mussels were absent, temperature and pH were significantly different between sites with and without freshwater mussels (Table 3.5). The results of the test would suggest that sites with higher pH and temperature and lower velocity measurements are associated with more mussels.

**Table 3.5: Differences in sites with mussels present and sites with mussels absent. Mean (± 1 SD) reported in table. Bolded values are statistically significant, α = 0.05.**

| | Sites with mussels ($n$ = 29) | Sites without mussels ($n$ = 11) | $p$-value |
|---|---|---|---|
| Temperature (°C) | 24.7 (2.31) | 21.4 (3.09) | **0.010** |
| pH | 7.37 (0.80) | 8.54 (0.79) | **0.009** |
| Dissolved Oxygen (mg/L) | 8.86 (2.64) | 8.83 (1.48) | 0.439 |
| Conductivity (µS/cm) | 112 (33.8) | 109 (50.2) | 0.763 |
| Turbidity (NTU) | 3.63 (3.49) | 3.57 (2.35) | 0.945 |
| Velocity (m/s) | 0.09 (0.02) | 0.13 (0.03) | 0.993 |
| Embeddedness | 35.6 (21.7) | 25.0 (22.0) | 0.551 |

Embeddedness, velocity, and pH were habitat variables correlated with several population metrics (Table 3.6). Temperature, turbidity, distance from upstream dam, and distance from downstream dam were other physical habitat variables that were correlated with select population metrics (Table 3.6).

**Table 3.6: Spearman rank correlation analysis reporting rho values. Bolded values represent results where the *p*-value < 0.05. Dashed line represents comparisons that could not be completed due to insufficient data. Where: *Ec* = *E. complanata*, *Lr* = *L. radiata*, *Pc* = *P. cataracta*, *Ai* = *Anodonta implicata*, *Au* = *Alasmidonta undulata*, *Lo* = *Leptodea ochracea*.**

| | Temperature (°C) | pH | Dissolved Oxygen (mg/L) | Conductivity (µS/cm) | Turbidity (NTU) | Velocity (m/s) | Embeddedness | Distance from US dam (km) | Distance from DS dam (km) |
|---|---|---|---|---|---|---|---|---|---|
| Species diversity | 0.14 | **-0.58** | -0.08 | 0.02 | -0.01 | **-0.47** | **0.53** | 0.39 | **-0.40** |
| Total CPUE | **0.34** | **-0.62** | 0.01 | -0.13 | -0.13 | **-0.37** | **0.48** | 0.32 | -0.31 |
| Total density | 0.29 | -0.08 | -0.08 | 0.26 | -0.11 | -0.07 | -0.12 | 0.23 | 0.71 |
| Species richness | 0.31 | **-0.69** | -0.03 | -0.14 | -0.07 | **-0.41** | **0.48** | 0.25 | -0.29 |
| Average length | -0.06 | **0.43** | 0.13 | -0.16 | 0.31 | 0.39 | -0.35 | -0.26 | -0.32 |
| *Ec* CPUE | **0.33** | **-0.58** | 0.01 | -0.17 | -0.08 | **-0.36** | **0.44** | 0.24 | -0.30 |
| *Lr* CPUE | 0.04 | **-0.53** | -0.06 | 0.04 | -0.06 | **-0.44** | **0.55** | 0.26 | **-0.40** |
| *Pc* CPUE | 0.26 | **-0.55** | -0.03 | -0.12 | -0.13 | **-0.37** | **0.52** | 0.33 | -0.36 |
| *Ai* CPUE | 0.10 | **-0.64** | -0.07 | -0.06 | -0.23 | **-0.35** | **0.43** | 0.05 | -0.07 |
| *Au* CPUE | 0.25 | -0.12 | 0.14 | -0.10 | **0.33** | -0.09 | 0.25 | 0.21 | -0.25 |
| *Lo* CPUE | 0.24 | -0.27 | -0.15 | 0.11 | -0.16 | -0.31 | **0.34** | **0.59** | 0.02 |
| *Ec* density | 0.17 | 0.16 | -0.02 | 0.19 | 0.01 | -0.02 | -0.24 | 0.15 | 0.71 |
| *Lr* density | **0.59** | 0.25 | -0.08 | 0.47 | 0.00 | 0.07 | 0.01 | **0.61** | 1.0 |
| *Pc* density | 0.28 | -0.32 | -0.22 | 0.21 | 0.09 | -0.20 | **0.84** | 0.29 | - |

The NMDS for species CPUE among sites grouped by physiographic region was used to compare species CPUE between the regions (stress = 0.05; Figure 3.2). The results suggest that the EH and CU regions have different species compositions based on the measured CPUE at each site. Excluding SJR26, the SJRV and NBL regions display overlap in species composition. The NMDS for physical habitat characteristics among sites grouped by freshwater mussel presence and absence was used to compare habitat composition between sites where mussels were and were not found during the surveys (stress = 0.0459; Figure 3.3). The results display overlap in the site-level physical habitat variables suggesting that these variables are not controlling factors in the presence/absence of freshwater mussels.

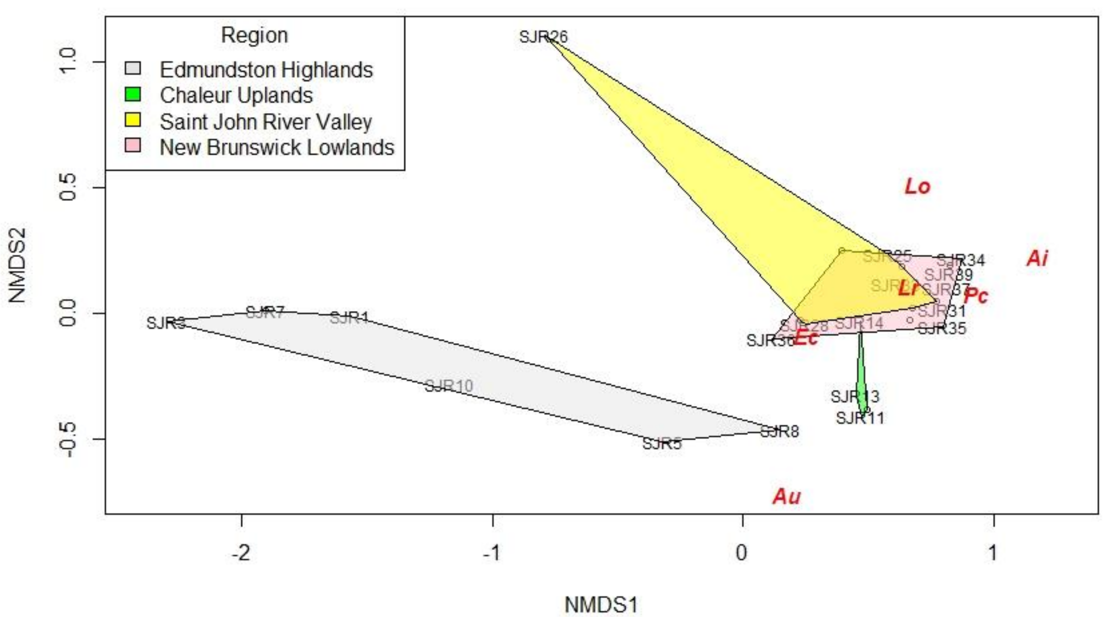


**Figure 3.2: NMDS plot of six species CPUE's (mussels/hour) among sites grouped by physiographic region: Edmundston Highlands, Chaleur Uplands, Saint John River Valley, and New Brunswick Lowlands (coloured grey, green, yellow, and red, respectively) and where *Ec = E. complanata, Lr = L. radiata, Pc = P. cataracta, Ai = A. implicata, Au = A. undulata, Lo = L. ochracea.***

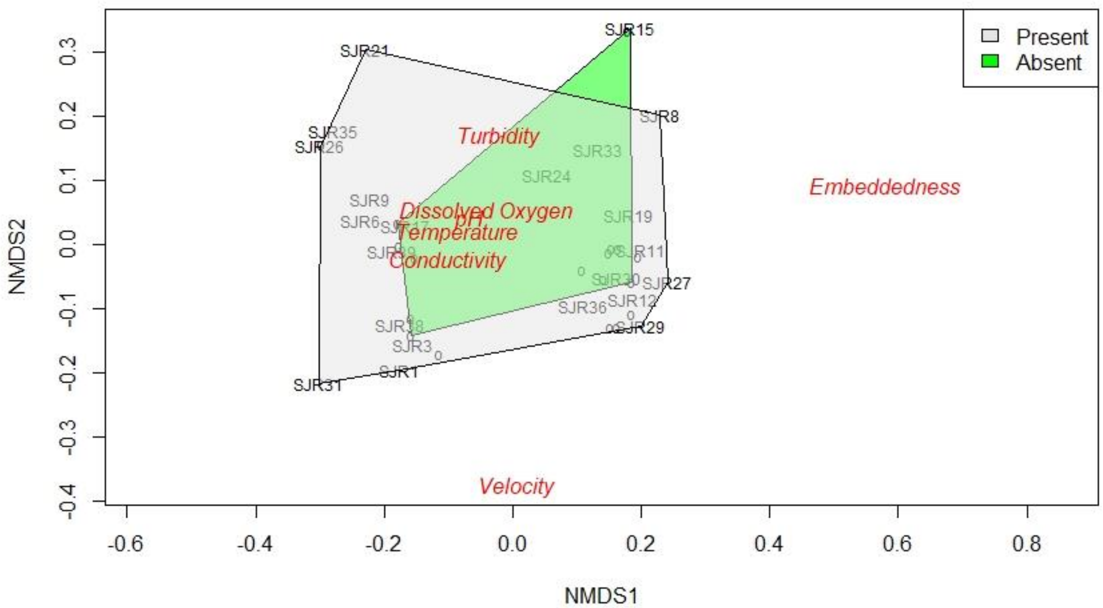


**Figure 3.3: NMDS plot of physical habitat characteristics measured at sites of freshwater mussel surveys grouped by presence and absence (grey and green, respectively). Physical habitat characteristics included temperature, dissolved oxygen, conductivity, velocity, turbidity, and embeddedness.**

## DISCUSSION

Freshwater mussels were found at 29 of the 40 sample sites chosen for this study. The CU region had the lowest rate of sites with mussels (40%), and the NBL region had the highest rate of sites with mussels (100%). Notable sites where mussels were absent include sites located in the GGS reservoir (SJR15 and SJR16) to sites 25 km downstream of the GGS, and sites between the BGS and 35 km downstream. Sites within a reservoir or 25-35 km downstream of the GGS and the BGS made up 9 of the 11 sites where mussels were not observed.

Sites where mussels were absent had lower water temperatures at the time of sampling compared to the sites where mussels were present. Sites SJR2 and SJR24 had water temperatures of 16°C on the day of sampling and were the coldest sites visited during the surveys (other sites had temperatures closer to 26°C at the time of sampling, comparatively). Both SJR2 and SJR24 are located directly downstream of outlets of cold-water streams (Crocs River and Becaguimec River, respectively). At these sites, cooler summer temperatures are likely an important factor controlling mussel distribution. Cooler temperatures stemming from the cold-water creeks would suppress mussel reproduction and deter warm-water fish hosts from these areas resulting in an absence of freshwater mussel communities (Heinricher & Layzer, 1999; Konrad et al., 2012). Sites located downstream of the GGS and BGS where mussels were absent (SJR17 to SJR20 and SJR22 to SJR24) also had lower temperatures (21°C) than sites located upstream of the GGS dam. Water temperatures can be significantly colder below and above a dam (Baxter, 1977; Galbraith & Vaughn, 2011); however, the cooler temperatures observed below the GGS and BGS dams persisted to 45 km and 38 km downstream, respectively, suggesting that the cooler temperatures may better reflect faster flow conditions in these sections of the river or cooler temperatures during the time of sampling. SJR21, located upstream approximately 20 km upstream of the BGS, had cooler temperatures consistent with other sites downstream of GGS and mussels were observed at this site. The presence of mussels at SJR21 indicates that temperature would not be the sole limiting factor of mussel presence for the sites located downstream of the GGS and the BGS.

The absence of mussels at sampling locations was also correlated with higher pH values. Hincks & Mackie (1997) showed that zebra mussel mortality was correlated with basic water (pH > 8.5) and low calcium concentrations ($< 25\ mg \cdot L^{-1}$) in laboratory studies. Calcium concentrations in the W|SJR would be considered low and range from 10 to 20 mg/L throughout the mainstem (New Brunswick Department of Environment and Local Government, 2019) and some sites had pH values > 8.5 during time of sampling. While possible that the relationship of high pH and low calcium with freshwater mussel mortality as seen in the Hincks and Mackie (1997) study may play a role in freshwater mussel distribution in the W|SJR, it is more likely that the higher pH values reflect the sites' local geology and substrate type which would control mussel distribution by creating suboptimal habitat conditions for freshwater mussels.

In general, freshwater mussel diversity, CPUE, and density increases while mussel size decreases, with downstream distance in the W|SJR. Mussel diversity was not statistically different between the EH and CU regions, and the SJRV and NBL regions, which demonstrates that the northern region above Grand Falls is compositionally different than the southern region below Grand Falls. This was also supported by the analysis of species CPUE composition among regions which indicated that the northern and southern regions each had distinctive species compositions. The NBL region had significantly higher CPUE and significantly smaller-sized mussels than the other regions. Mussel density was not significantly different between the CU, SJRV and NBL regions, suggesting that mussel densities in mussel beds (areas of moderate to high mussel density) were similar across the regions.

Physical habitat characteristics grouped by the presence or absence of mussels at the site level showed significant overlap in the physical parameters measured at sites with and without mussels. This suggests that the physical variables measured at the site scale were poor predictors of freshwater mussel presence and absence in the W|SJR. While this result could be a reflection of the distribution or sample size of sampling sites, mixed conclusions have been found when using site-level habitat variables to describe or predict freshwater mussel distribution and occurrence (Strayer and Ralley, 1993; Layzer and Madison, 1995; Haag and Warren, 1997; Strayer, 2008), and it is generally accepted that site-level habitat variables are factors in mussel distribution but are likely not the controlling factors (Pandolfo et al., 2016). The relationship between mussels and microhabitat variables is further complicated by the fact that site-level habitat variables are usually a reflection of habitat at a larger scale (Pandolfo et al., 2016). The results of my study support those of others which suggest that freshwater mussel distribution and abundance is more easily predictable at scales of kilometres, rather than scales of metres (Strayer, 1983; Strayer, 1993; Strayer and Ralley, 1993; Strayer et al., 1999; Haag, 2012). Studies completed by Layzer and Madison (1995), Hardison and Layzer (2001), Zigler et al. (2008), and Allen and Vaughn (2010), have suggested that hydraulic variables such as substrate stability and shear stress are better predictors of freshwater mussel distribution than traditional microhabitat variables. Increasing the number of sites and sampling more variable habitat (including areas that might be considered suboptimal mussel habitat) would help to confirm the relationships observed between mussel metrics and site-level physical habitat variables.

Some of the macrohabitat variables that best describe freshwater mussel species diversity and abundance include climate, glacial history (which can include barriers to dispersal and fish host distribution), geology, and proximity to an impoundment (Strayer et al., 1999; Haag, 2012). Climate is thought to be a limiting factor for some mussel species in more northern climates (Clarke, 1973; Harper et al., 2012). Mussels are generally absent in areas where mean July air temperatures are lower than 15 – 18 °C (Clarke, 1973). Colder air may result in water temperatures that are too cold to support the physiological requirements necessary for reproduction and survival, and ice scour may negatively impact the persistence of adult mussels (Burlakova et al., 2011; Harper et al., 2012). Climate may be a limiting factor in more northern areas of the W|SJR given the higher latitude (difference in two degrees between most northern and most southern sites) and colder average winter temperatures. However, the mean summer temperatures at both northern and southern sites would be warmer than the 15 – 18 °C limit specified by Clarke (1973; Government of Canada, 2021).

Glacial history is also thought to be a strong determinant of freshwater mussel distributions (Strayer, 1983). Glacial retreat after the last ice age would have limited the dispersal of freshwater fish hosts into Atlantic Canada (Curry, 2007). Glacial retreat also changed and influenced regional geologies, which is considered a crucial environmental factor controlling mussel distributions (Strayer, 1983; Mcrae et al., 2004). The present-day path of the W|SJR flows over Grand Falls; however, this was not its historical path (Curry, 2007). The historical path of the W|SJR limited the dispersal of freshwater fishes and that would have meant limiting the dispersal of freshwater mussels as well (Curry, 2007).

Geology influences discharge which in turn affects other habitat variables such as water velocity (Ledger, 1981), substrate type (Leopold et al., 2020), variability in water temperature (van Vliet et al., 2013), water chemistry (Strayer, 1983), and habitat stability (Johnson et al., 1997; Mcrae et al., 2004). These habitat variables directly affect the availability of food particles in both the water column and the sediment (Rypel et al.,

2009) and affect all mussel life stages (glochidial production and development; juvenile settlement, growth, and survival; adult movement, reproduction, and survival).

The construction of a dam creates difficult conditions for freshwater mussels and other aquatic organisms to persist upstream and downstream of the impoundment. Reservoirs readily accumulate fine sediments which increase embeddedness and can clog the interstitial spaces used by both adult and juvenile mussels (Baxter, 1977; McMahon & Bogan, 2001). Stranding in reservoirs can be common since reservoirs are drawn down depending on hydropower demand and are occasionally drained for dam repairs or maintenance (Richardson et al., 2002; Fisher & Lavoy, 2011). The reach of river downstream of a dam can experience artificially fast flows (known to scour away both mussel beds and smaller riverbed substrates required by most mussel species) which generally results in larger sediments that may not be able to support successful mussel populations (Payne & Miller, 1987; Vaughn & Taylor, 1999). Any reach of river downstream of a dam being used for hydropower is going to be a highly unstable environment due to daily hydropeaking activities and water level fluctuations (Poff et al., 1997; Watters, 1992). The presence of the BGS is likely a limiting factor for mussel populations in this reach of the river since no freshwater mussels were encountered within 35 km of the dam.

Species diversity was significantly different between the northern region and the southern region of the W|SJR, which are delineated by Grand Falls, a natural barrier to fish dispersal and migration. This is evidence that mussel diversity in the W|SJR is controlled largely by the region’s glacial history and fish host distribution. Previous research has shown that Atlantic slope mussel species are limited less by the distribution of fish hosts and more by the presence of dispersal barriers (Haag, 2010, 2012).

The highest CPUE was found in the southern-most region in the W|SJR, which corresponds with the portion of the river with the greatest area. Higher abundance in downstream river reaches has been found in other studies (e.g., van der Schalie, 1938; Rahel & Hubert, 1991; McCabe, 2011; Randklev et al., 2016) on large streams and rivers and is likely explained by the species-area relationship (Rahel & Hubert, 1991; Watters, 1992; Haag, 2012). Positive correlations between abundance and area likely depend on the presence of large areas of suitable habitat for that species (Watters, 1992; Connor et al., 2000). Larger areas of the W|SJR present in the lower reach may be able to support greater abundance of freshwater mussels due to a greater amount of ideal, stable habitat (Allan, 2004). Where more suitable habitat exists for freshwater mussels, it also exists for their host species resulting in more diverse and abundant fish host populations. Limiting factors to mussel abundance are similar to those that limit species diversity at a regional scale (climate, glacial history, and geology), and differences in local habitat variables such as complex hydraulic variables (e.g., shear stress) and substrate stability likely have strong influence on local mussel abundance (Layzer & Madison, 1995; Hardison & Layzer, 2001; Allen & Vaughn, 2010). The investigation of the relationship between freshwater mussels and hydraulic variables in the W|SJR is something that should be considered for a better understanding of the mussel-habitat relationship.

*Ecological Implications*

The results of this study show that the freshwater mussels in the W|SJR have significant relationships with their physical habitat, and much of their distribution and abundance can be explained by factors such as regional geology, barriers to dispersal, and anthropogenic impacts. Site-level habitat variables were poor predictors of freshwater mussel presence and absence. Species diversity and composition is best delineated by Grand Falls, a natural barrier to mussel and fish dispersal. Mussel abundance generally increased with downstream distance, most likely the result of an increasing availability of fine sediment habitat in the lower W|SJR. Ideal long-term monitoring sites should have a high abundance of mussels relative to other sampling locations and would represent self-sustaining, stronghold populations (Randklev et al., 2018). In each region of the W|SJR, we found sites that could be used as long-term monitoring locations in the future. *E. complanata* was in high abundance at sites in all regions of the W|SJR and would be an ideal sentinel species to assess environmental change in the river.

Long-term habitat quality and stability are keys to freshwater mussel persistence and survival. Aquatic habitat in the W|SJR will continue to change with time, and threats such as the introduction of invasive species, climate change, agricultural runoff, and urbanization may result in altered freshwater mussel habitat and jeopardize currently stable populations.

Limited historical data for the W|SJR makes detecting population trends or changes extremely difficult and spurious at best. The preliminary data collected during this baseline assessment provides information that will help guide freshwater mussel management and conservation (including guiding potential relocation efforts) and should be used to make informed decisions regarding future projects in the W|SJR. Most importantly, this study serves as a foundation for continuing freshwater mussel research. Further studies are necessary to better understand the possible relationships between freshwater mussel populations in the W|SJR and physical habitat, as well as the effects of anthropogenic factors on their distribution and abundance.

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# Chapter 4 - Summary

Freshwater mussels are recognized as one of the most imperiled groups of fauna on the planet (see for example: Bogan, 1993; Williams et al., 1993; Collier et al., 2016). They are highly sensitive to changes in their environment and have been overlooked in terms of conservation efforts until more recent decades and as such, populations around the world have experienced loss of freshwater mussel diversity and abundance (e.g., Dudgeon et al., 2006; Vaughn, 2010; Haag & Williams, 2014). In an attempt to recover and protect freshwater mussels, exploratory surveys and monitoring programs are being used in Canada and around the world to better define mussel distributions and to assess population trends.

In New Brunswick, freshwater mussel surveys and published freshwater mussel data are few and limited resulting in a lack of complete historical and current data for freshwater mussel populations in the province (Hanson & Locke, 2001; Sabine et al., 2004; Sollows et al., 2013). I executed an exploratory, baseline assessment of freshwater mussel assemblages across 360 km of the W|SJR. Using both quantitative and semi-quantitative survey methods, I was able to characterize freshwater mussel diversity, abundance, and density throughout the surveyed section of the river and gathered valuable information pertaining to mussel size, juvenile presence/recent recruitment, and an important species at risk. I also measured and collected physical habitat data that I was able to use to assess relationships between freshwater mussels and their environment.

In Chapter 2, I characterized mussel diversity, abundance, and density in four defined physiographic regions of the W|SJR. I found that diversity and abundance in the

W|SJR followed the pattern of continuous variation as well as the species-area relationship, whereby diversity and abundance increased with downstream distance in the river. Other studies have found similar patterns in freshwater mussel diversity and abundance in fluvial systems (e.g., van der Schalie, 1938; Rahel & Hubert, 1991; McCabe, 2011; Randklev et al., 2016). The observed distribution of mussels in the W|SJR was also a function of species-specific ecologies, habitat conditions and availability and dispersal barriers.

Finer substrates and more stable habitat in the lower W|SJR (the NBL region) provided ideal habitat for endobenthic and juvenile freshwater mussels. Evidence of burrowed mussels and a low count of juvenile mussels downstream of the MGS may indicate significant dam effects on populations subjected to fluctuating water levels. Burrowing behaviour and lower recruitment rates downstream of dams is common due to adverse environmental conditions in otherwise favourable substrates (Vaughn & Taylor, 1999; Schwalb & Pusch, 2007). The presence of juvenile *E. complanata*, *L. radiata*, and *P. cataracta* in the NBL region suggests that recruitment of these species is actively occurring in this region and these species populations would be considered stable. I found a concerning and pronounced decline in *L. cariosa* presence and abundance at sites considered to have medium to high *L. cariosa* abundance when last surveyed in 2001 (Sabine et al., 2004). This decline has been noticed by other scientists in more recent years and *L. cariosa* individuals have been absent in locations where they used to be abundant in the past (K. White, personal communication, 2018). The reason for the decline is unknown, but the leading cause is theorized to be periods of low water level and elevated water temperatures in the late summer months during dry years (Committee on the Status of Endangered Wildlife in Canada, 2004; D. McAlpine, pers. comm., 2018). Periods of drought such as these have been known to cause die-off events due to stranding and intolerable habitat conditions (Allen et al., 2013; DuBose et al., 2019).

In Chapter 3, I examined relationships between freshwater mussels and physical habitat variables. I found that compositionally the W|SJR can be split into two distinct areas (northern and southern) where similar species compositions occur. The two areas are divided by Grand Falls, with the northern area being upstream of Grand Falls (the EH and CU regions) and the southern area being downstream of Grand Falls (the SJRV and NBL regions). Freshwater mussel density was not significantly different between regions suggesting that mussel bed densities are similar throughout the river. The NBL region had significantly higher freshwater mussel abundance and diversity compared to the other regions.

The results show that the distribution and abundance of freshwater mussels in the W|SJR can largely be explained by factors such as the proximity to an upstream or downstream dam, local geology, and barriers to dispersal. Variables measured at the site-level were determined to be poor predictors of freshwater mussel occurrence in the W|SJR. Some sites identified in my study (such as SJR13, SJR25, SJR28, SJR32, SJR37) would be considered ideal long-term monitoring sites due to their greater abundance and higher diversity of mussels, and *E. complanata* would be a good candidate for a sentinel species that can be used to assess environmental change in the river. A more extensive survey could be completed at SJR31 in attempt to locate more *L. cariosa* individuals.

*Final Synopsis*

Our current understanding of freshwater mussels in the W|SJR and the province of New Brunswick is incomplete and more exploratory surveys and coordinated monitoring programs are necessary to collect accurate and representative population data. Species at risk in Canada are protected by SARA, however SARA needs up to date and representative data to effectively identify species that need protection and to properly manage identified species at risk. Management goals for research and monitoring established by SARA for *L. cariosa* were not met for the 2010-2015 period in the W|SJR, which indicates an immediate need for updated *L. cariosa* population estimates (Environment and Climate Change Canada, 2017). Prior to completing my study, *L. cariosa* populations were considered stable in the W|SJR (Sabine et al., 2004). The results of my study identify the need for more frequent monitoring programs to provide appropriate protection to species that need it most.

Future recommendations for freshwater mussel research in the W|SJR would include:

- Surveys for rare mussel species (e.g., *L. cariosa* and *L. ochracea*) to update population estimates and to better prioritize management goals,
- Sampling new, potentially mussel-bearing sites with greater replication for more accurate density estimates,

- Collection of complex hydraulic variables to investigate the relationship between freshwater mussels and substrate stability, and shear stress,
- Visual surveys in unexplored areas of the river,
- Surveys at depths > 2 m,
- Execution of surveys in the MGS headpond to search for potential *L. cariosa* populations, and
- Identify fish hosts for the freshwater mussel species of the W|SJR, many of which are still unknown or unconfirmed.

My baseline assessment provided initial estimates and information for freshwater mussel populations in the W|SJR and identified knowledge gaps and potential future steps. There is opportunity for a more well-targeted, concerted effort with freshwater mussel research in the W|SJR and New Brunswick which would help to improve the identification, management, and protection of at-risk species, as well as freshwater mussel populations as a whole.

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